# Understanding trait-based community assembly in tropical savannahs at different trophic levels

van der Plas, Alfons Leendert Derk

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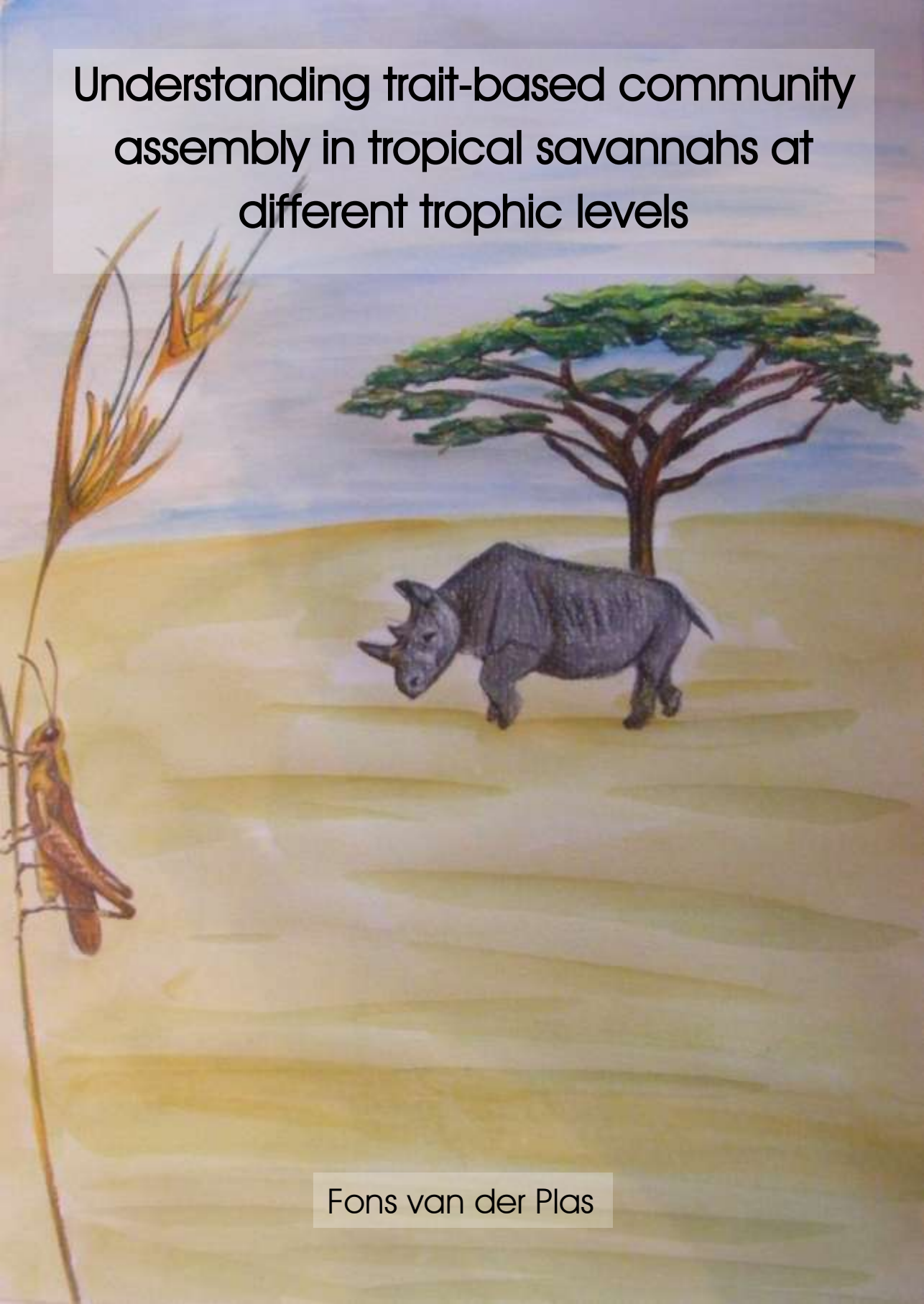

Fons van der Plas

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# Understanding trait-based Community assembly in tropical savannahs at different trophic levels

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# Contents

# Chapter 1: General introduction

Fons van der Plas

Understanding by which processes ecological communities are assembled is a central goal in ecology. Some of the earliest studies investigating community assembly (Clements 1916; Phillips 1931) noted that the formation of ecological communities is "straightforward" and highly deterministic, consistently following certain trajectories based on shared ecological requirements among groups of species. In sharp contrast with this, another pioneering study on community assembly stated that "… an [species] association is … merely a coincidence" (Gleason 1926). These different views inspired two different 'camps' among researchers on community assembly: those who emphasize deterministic, niche-based processes driving community assembly (e.g. Hutchinson 1959; Diamond 1975; Tilman 1982) and those who emphasize the importance of stochastic or random processes in community assembly (e.g. Caswell 1976; Sale 1977; Hubbell 2001). Furthermore, the 'niche-based camp' is itself composed of different views: while some researchers emphasize competition as the main structuring force in community ecology (e.g. Tilman 1982), others additionally take other niche-based processes into consideration, such a positive species interactions (Bertness & Callaway 1994) and stress-based filtering (Weiher & Keddy 1995a). Nowadays, it is increasingly recognized that it is a combination of different deterministic, niche-based *and* stochastic processes shaping ecological communities (Vellend 2010; Weiher et al. 2011; Rosindell et al. 2011), but it is still largely unknown how the complex interplay between these different processes shapes communities.

## 1.1 Traits and community assembly

One dominant approach to study community assembly is the analysis of how functional traits are distributed in communities of co-occurring species. A trait is usually defined as a measurable property of an organism, and considered functional if it has an impact on the performance of the individual it belongs to (McGill et al. 2006). In other words, a functional trait is related to the niche of a species, although some traits are more so than others. Researchers often distinguish between 'hard' functional traits that are usually physiological and highly related to a species niche (e.g. relative growth rate, metabolic rate, photosynthetic capacity, transpiration, etc.) and 'soft' morphological or chemical (e.g. body size, leaf area, leaf nitrogen content) traits (Fig. 1.1A). While physiological traits are usually more closely related to a species niche, they are not as easy to measure as morphological or chemical traits, especially in the field. Fortunately, numerous studies have investigated relationships between 'soft' morphological or chemical traits and 'hard' physiological traits, which are in many cases reasonably strong (e.g. Kleiber 1947; Poorter et al. 1990; Wright et al. 2004; Chave et al. 2009), making these morphological and chemical traits suitable proxy's describing part of a species' niche. Consequently, these 'soft' traits are the traits that are usually measured to study community assembly in the field, with two main goals: to understand (1) how species' occurrences respond to environmental gradients and (2) to understand which species can potentially co-occur.

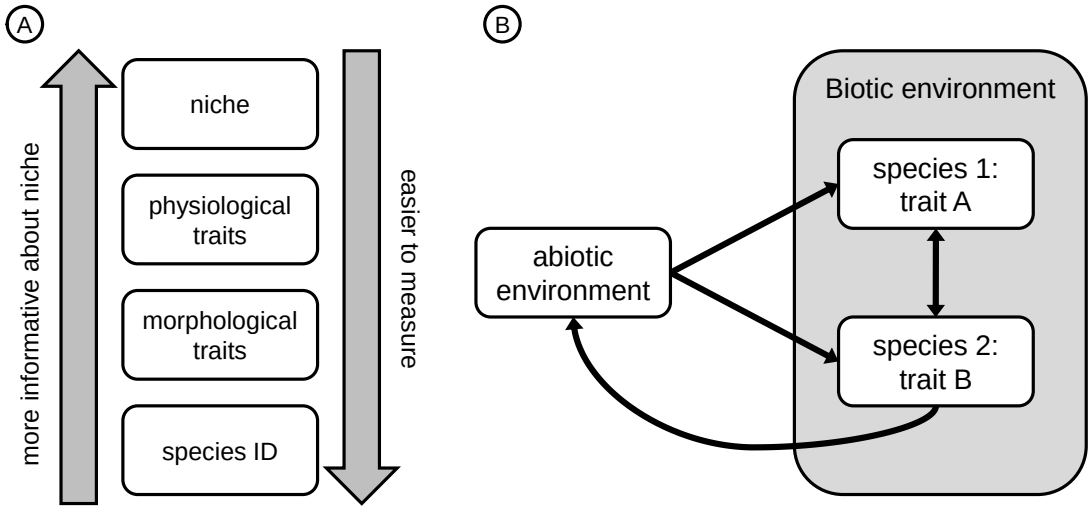


**Figure 1.1.** A. An overview of different properties of a species and the information they give about the species' niche and their difficulty to measure. B. Relationships between the abiotic environment, biotic environment and the (co)occurrence of species with different traits.

### 1.1.1 Functional traits and occurrences of species

Species differences in functional traits usually reflect species differences in their position along a continuous life-history trade-off. For example, plants with thin leaves (high Specific Leaf Area or SLA) are usually more efficient in capturing light and in using this to make sugars (Wright et al. 2004) and consequently have a higher growth rate than species with thicker leaves (Poorter et al. 1990; Reich et al. 1992). While this may seem as a big advantage, the flipside is that these thin leaves usually also have a lower lifespan than more robust, thick leaves (Grime et al. 1997; Reich et al. 1992; 1997). Consequently, a high SLA is usually most advantageous in environments with many resources (water, light, nutrients) (Ordonez et al. 2009), where plants can quickly 'pay off' (i.e. photosynthesize) their investment (construction of the leaf), before the leaf dies. In environments where resources are scarce, more efficient leaves hardly make a plant grow faster, due to the shortage of resources, and consequently there it is more advantageous to have long-living, but less efficient leaves. Such life-history trade-offs are ubiquitous in nature: others include trade-offs between investment in seed size or in the number of seeds (Kakobsson & Eriksson 2000) and between resource demands and predation risk, reflected by differences in body size (Houston et al. 1993, Hopcraft et al. 2010). Due to these kind of trade-offs, there is thus (1) variation between species in functional traits, and (2) these functional traits can often predict in which type of environment (e.g. high rainfall / low rainfall) a species can or cannot occur (Fig. 1.1B).

### 1.1.2 Functional traits and co-occurrences between species

If the assembly of communities is *only* affected by differences between species in coping with abiotic conditions (hereafter: abiotic filtering) and if there is only one main strategy (i.e., set of traits) to adapt to these abiotic conditions, then we would expect that in a certain environment, co-occurring species would be rather similar to each other (Weiher & Keddy 1995a; Webb et al. 2002; Weiher et al. 2011). However, usually nature is more complex. Species not only respond to their abiotic environment, but there is also a feedback (Fig. 1.1B) and thereby species can have indirect effects on the occurrence of other species (Jones et al. 1994). Additionally, species are often directly affecting other species and being affected by other species (Fig. 1.1B), through e.g. trophic interactions, competition, facilitative

and mutualistic interactions. Such biotic interactions might alter expectations on trait distributions of co-occurring species. Although there are many types of interactions between species, only one has so far gained much attention in literature about trait-based community assembly: competition.
Negative species interactions are, at least in plants, ubiquitous in ecology (Tilman 1982). In many cases, such negative species interactions arise from competition between species for shared resources. This notion was already recognized by Darwin and considered by him as a driving force for natural selection (1959). Less well known is that in his famous book, Darwin also stated one of the first ecological hypotheses, namely that (phylogenetically or functionally) similar species compete more strongly with each other than more dissimilar species, due to their shared adaptations (traits) for a certain resource. If this is true, then one might also expect that high trait similarity between species leads to competitive exclusion, and that as a consequence, sets of species need to be sufficiently *dissimilar* in order to co-occur (Hutchinson 1959; Weiher & Keddy 1995a; Webb et al. 2002).
We thus see that while species need to be similar to each other to cope with certain abiotic conditions, at the same time, when resources become limiting, too much similarity will lead to competitive exclusion. The question is what will happen with trait distribution patterns of co-occurring species when both abiotic filtering *and* competition act upon community assembly. Usually, researchers expect that due to the need to cope with local abiotic conditions, only species with trait values that fall within a certain *range* will be able to occur in a particular environment (Weiher & Keddy 1995a; Cavender-Bares et al. 2004; Cornwell & Ackerly 2009). As a consequence, this should lead to underexpansion: a lower trait range than expected under 'random assembly' (i.e. a random set of species, with the same species richness, Fig. 1.2A) (Weiher & Keddy 1995a; Cavender-Bares et al. 2004; Cornwell & Ackerly 2009). Of the species that are able to deal with the local environment, those species that are too similar to each other will compete strongly, leading to the exclusion of some species, with as a result a high *evenness* in adjacent trait values *distances* (Weiher & Keddy 1995a; Cavender-Bares et al. 2004; Kraft et al. 2008; Cornwell & Ackerly 2009, Fig. 1.2B).

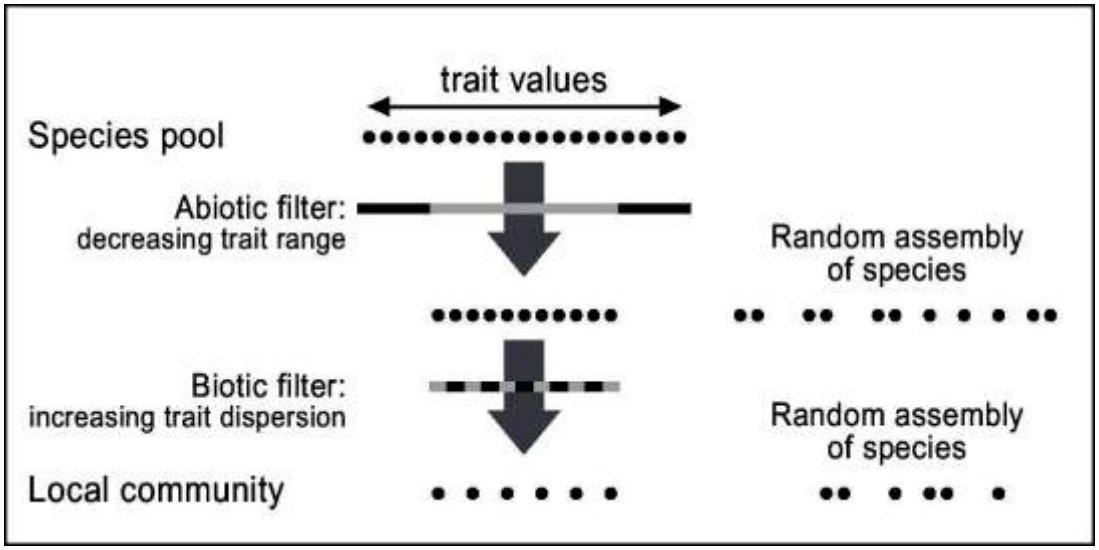


**Figure 1.2.** A classical idea of community assembly. Here, when abiotic filters drive community assembly, only species with quitable traits are able to live in a local environment, leading to trait underexpansion patterns. 'Biotic filters', usually used to label competition, should lead to limiting similarity of shared resources between co-occurring species, and therefore to trait overdispersion patterns.

Although this view on community assembly is attractively simple and intuitive, it has also been criticized. Firstly, the hypothesized effects of abiotic filtering and competition on trait distributions of co-occurring species can be different when certain assumptions are not met (Marks & Lechowitz 2006; Mayfield & Levine 2010). Perhaps even more importantly, many ecological processes, such as predation, facilitation and ecosystem-engineering, have been almost totally ignored in literature on trait-based community assembly. It therefore remains the question how our view on community assembly changes when incorporating these. This thesis handles with three main questions: (1) how can trophic interactions and (2) ecosystem-engineering processes affect trait distributions of (co-)occurring species and (3) what do observed trait distribution patterns of co-occurring species tell us about the relative contribution of different types of community assembly processes?

## 1.2 Study Systems

Studying community assembly can best be done in systems with high habitat heterogeneity: those are systems that contain very different communities, and therefore many potential 'replicates' to study. Savannahs are well known for their diversity of different vegetation types (Scholes 1993). By definition, they consist of two co-dominant growth forms: trees and C4 grasses. Additionally, the grass layer can usually be subdivided in two grassland types: grazing lawns, consisting of horizontally growing and mostly clonally reproducing lawn grass species, and bunch grasslands, consisting of vertically growing, mostly sexually reproducing bunch grass species (McNaughton 1983; Cromsigt & Olff 2007). Partially due to this co-dominance of different vegetation types, African savannahs are incredibly diverse (Mittermeier et al. 1998). This raises the question why these different vegetation types can co-exist. Numerous hypotheses have been put forward: due to large rainfall gradients, spatial variation in fire frequencies, soil fertility and structure gradients, and due to smaller-scale, biotically (e.g. by herbivores and termites) driven heterogeneity (McNaughton 1983,1985; Augustine & McNaughton 1998; Archibald et al. 2005; Cromsigt & Olff 2008; Waldram et al. 2008; Moe et al. 2009; Stock et al. 2010; Anderson et al. 2011; Gosling et al. 2012). Most of the hypotheses emphasizing local scale heterogeneity (e.g. Archibald et al. 2005; Cromsigt & Olff 2008; Waldram et al. 2008; Gosling et al. 2012) come from studies performed in Southern Africa, while most hypotheses emphasizing drivers of larger scale heterogeneity (e.g. McNaughton 1983; Anderson et al. 2011, Hopcraft et al. 2012) come from studies performed in Eastern Africa. This is not surprising, since these regions differ remarkably. While high habitat heterogeneity and biodiversity is observed in both subcontinents, it is mostly observed at larger scales in eastern Africa, while mosaics of vegetation patterns and their associated biodiversity can already be seen at very small scales in Southern Africa (Cromsigt & Olff 2008). It is for this reason that the research of this thesis was performed in two different African parks (Fig. 1.3): Hluhluwe-iMfolozi Park (HiP) in South Africa, where small scale gradients in elevation and soil types, in combination with biotic agents (herbivores, termites) promote local scale heterogeneity (Archibald et al. 2005; Cromsigt & Olff 2008; Waldram et al. 2008; Gosling et al. 2012), and Serengeti National Park (SNP) in Tanzania, where it is mostly rainfall (Sinclair 1979), but also termites and rocky outcrops that drive the spatial turnover of different vegetation types (McNaughton 1984,1985; Anderson et al. 2011; Poelchau & Mistry 2011).

## 1.3 Outline of this thesis

### 1.3.1 Trophic interactions and community assembly

All animals are consumers, eating either other animals, plants detritus. Additionally, all plant and animal species are at least during some part of their development vulnerable to predation. It therefore seems rather trivial that trophic interactions should be important in understanding community assembly. Despite this, the literature on trait-based community assembly hardly pays any attention to these universal processes, possibly caused by the strong bias in the literature towards plant studies,

where competition is a classic study subject. This leaves the question open how both bottom-up and top-down processes can affect trait distribution patterns of co-occurring species.
When consumers are at least to a certain extent specialistic in their diet, eating only one or a few (similar) species, one might expect that certain trait distribution patterns of prey-communities can be imprinted on consumer communities. For example, if a plant community only exists of a group of phylogenetically similar species which all produce the same type of alkaloid, then all species from the herbivore community living from these plant species should have similar traits to cope with the toxic alkaloids. Similarly, in African savannahs were large predator species usually also consume larger prey species (Owen-Smith & Mills 2008), one might expect that size distribution patterns of prey species can have bottom-up effects on size distribution patterns of predator species.
Different patterns might be expected if top-down processes are more important in community assembly. When consumer communities are dominated by species that have specialistic diets, eating a few (similar) species, one might expect that this leads to limiting similarity for predation risk: consumption of prey might lead to the extinction of all but one prey species (apparent competition), resulting in limiting similarity of anti-herbivory traits or traits related to nutritiousness of prey (Abrams 1983). Alternatively, when consumer species have more generalistic diets, one might expect quite different effects. Having a generalistic diet usually does not mean that consumers are totally not selective: for example, ungulates with quite broad diets (Crawley 1983) still tend to prefer plant species that are relatively rich in nitrogen. In such cases, consumers are expected to constrain trait variation among prey species: either prey species will not profit from grazing, in which case grazing will select for a community only consisting of nitrogen poor species, or prey species do profit from grazing, in which grazing will select for a community only consisting of nitrogen-rich species.
We tested these ideas in the first four chapters. Specifically, we asked (1) how the grazing of rather generalist (ungulate) herbivores affects trait and diversity patterns of grasses and insects; (2) whether grasshopper trait assembly is bottom-up regulated by plant trait assembly, or vice versa and (3) how the species and functional composition of tree and browser communities respond to both small scale and large scale sources of heterogeneity and to variation in predation risk.

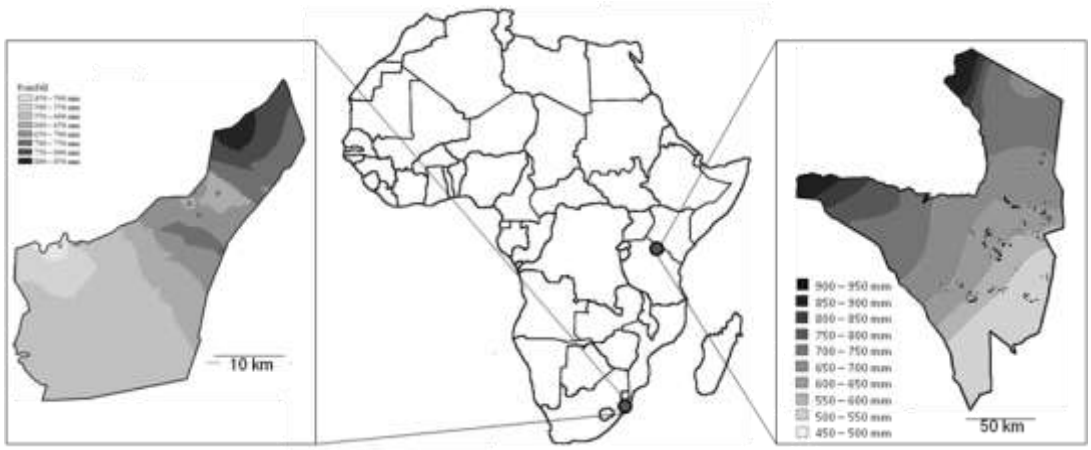


**Figure 1.3.** Rainfall maps of the two research areas visited for this thesis (left and right), plus a map of Africa with the locations of both research areas. The left map shows Hluhluwe-iMfolozi Park (HiP), a small savannah park in South-Africa, which despite its size harbors a high habitat heterogeneity, partially due to the steep gradient in rainfall. The right map shows Serengeti National Park (SNP), located in Tanzania. SNP is a much larger park with a similar range in rainfall, although this gradient spans over a much larger area.

### 1.3.2 Ecosystem-engineering and trait-based community assembly

Ecosystem-engineers are organisms that affect populations of communities of other species by altering the availability of resources or by changing abiotic conditions (Jones et al. 1994). Although almost all species can be considered ecosystem-engineers, the term 'ecosystem engineering' is nonetheless useful for describing the non-trophic interactions by which an organism affects other species positively or negatively (Laland et al. 1999). Examples of ecosystem-engineering include the construction of beaver dams, mound building activities of termites and ants, the effects of trees on microtemperatures in their shade and the soil compaction effects as a result of trampling by large herbivores. This list of examples illustrates that ecosystem-engineering includes a large variety of effects, and therefore one should not expect that effects of these examples of ecosystem-engineering on community assembly are consistently the same.

In cases where ecosystem-engineering processes by a species are 'relaxing' harsh environmental conditions for other species, one might expect ecosystem-engineering to oppose the underexpansion effects of 'abiotic filtering'. This might for example happen on termite mounds, which have much more fertile, moist soils than their surroundings. On the contrary, in other cases ecosystem-engineering can be expected to create conditions that are stressful for other species. For example, the trampling of herbivores can reduce oxygen levels in salt marshes (Schrama et al. 2013) or soil moisture in arid systems (Belsky 1986), increasing abiotic stress. In such cases, one might expect ecosystem-engineering to cause trait underexpansion patterns.

We tested both these ideas. Specifically, we investigated the effects of one ecosystem-engineer that is expected to reduce abiotic stress (mound-building termites benefitting trees) and of another one that is expected to increase abiotic stress (ungulates constraining grasses). For mound-building termites, we expected that due to the increased moisture and nutrient levels found in mound soils (Holt & Lepage 2000; Gosling et al. 2012), in combination with the lower fire frequencies (as suggested by Dangerfield et al 1998), abiotic stress would be reduced on mounds and that this would increase trait variation within tree communities. For ungulates, we expected that their trampling would compact soils, thereby reducing water infiltration and increasing abiotic stress for grasses. Therefore, we tested the effects of drought on different functional types of savannah grass species.

### 1.3.3. Putting everything together: is community assembly a mess?

As stressed earlier, community assembly can be driven by the interplay of a large number of different ecological processes. While for a full understanding of community assembly a recognition of its great complexity is necessary, there is also the risk that such an 'everything is related with everything' view will obscure us from understanding the 'bigger picture', only rendering us to view community ecology as a "mess", sensu Lawton (1999). In the last research chapter, we propose a new way to look at community assembly, summarizing all different community assembly processes into three different categories: processes which constrain trait space of co-occurring species, processes that limit trait similarity of co-occurring species and community assembly processes that are not mediated by traits. We then demonstrate a modeling approach to estimate the relative contributions of these three different categories of community assembly processes, and apply it to savannah tree communities.

## Section I: Bottom-up and top-down effects in community assembly

# Chapter 2: Mesoherbivores affect grasshopper communities in a megaherbivore-dominated South African savannah.

Fons van der Plas & Han Olff

## Abstract

African savannahs are among the few places on earth where species rich communities of ungulate grazers dominate ecosystem functioning. Less conspicuous, but even more diverse, are the communities of herbivorous insects that also consume significant, large amounts of biomass. Various studies investigated the community assembly of these separate groups, but it is poorly known how these potentially competing guilds interact. Ungulates can affect herbivorous insects through reducing vegetation height, promoting plant quality and altering plant community composition, but different effects are expected for different-sized species.

Here, in a South African savannah, we investigated to which extent ungulate species of different size differ in their effects on grasshopper communities. White rhino are the most abundant vertebrate herbivore (in biomass/ha) in our study site, while also various mesoherbivores commonly occur, such as buffalo, zebra and impala. In an exclosure experiment, at eight sites we compared their effects on grasshopper communities using three nested treatments: i) unfenced plots ('megaherbivore grazing') with all vertebrate herbivores (largest to small) present, ii) plots with a low cable fence, excluding white rhino ('mesoherbivore grazing'), and iii) plots with tall, small-mesh fences, additionally excluding all other vertebrate herbivores larger than rodents ('microherbivore grazing'). In each treatment, we collected data of vegetation height, grass and grasshopper community composition, and several functional traits of both grasses and grasshoppers.

We found that mesoherbivores altered vegetation characteristics and plant and grasshopper communities, while megaherbivores did not. Plots without mesoherbivores contained higher vegetation with different grass and grasshopper species than mesoherbivore or megaherbivore-grazed plots. These microherbivore-grazed plots hosted larger grasshopper species, suggesting that mesoherbivores excluded these. Further analyses showed a strong correlation between average body size of grasshoppers and vegetation height and also a strong correspondence between grass and grasshopper communities structure, suggesting by which mechanisms mesoherbivores affect grasshopper communities.

## 2.1 Introduction

African savannahs are famous for their high diversity and abundances of large ungulate grazers, ranging from small antelopes to rhinos and elephants (Prins & Olff 1998). Although less conspicuous, insects are even more abundant and diverse in these ecosystems (Samways 1999). Some of these insects share resources with ungulates: plants. Grasshoppers are usually considered as the most important group of herbivorous insects in savannahs, consuming a substantial part of the plant biomass (Sinclair 1975). Additionally, like most insect groups, grasshoppers form species rich communities (Sinclair 1975; Gebeyehu & Samways 2003). Due to the importance of both ungulate and grasshopper communities for savannah functioning, it is no surprise that several studies have investigated the community assembly of these groups (Olff et al. 2002; Gebeyehu & Samways 2003; Arsenault & Owen-Smith 2002, Cromsigt et al. 2009; Kleynhans et al. 2011; Van der Plas et al. 2012). However, these studies all investigated community assembly of ungulates or grasshoppers in isolation, although these different guilds share similar resources and therefore potentially compete.

Ungulates, but not insects, generally exert strong top-down effects on the vegetation (Crawley 1989). Consequently, one could expect ungulates to have similarly strong effects on the community assembly of grasshoppers, since these are usually bottom-up regulated by plant communities (Ritchie 2000; Joern 2005; Van der Plas et al. 2012). Studies experimentally excluding ungulate grazing in

savannahs or similar ecosystems indeed support this expectation (Prendini et al. 1996; Gebeyehu & Samways 2003; Joern 2005). However, these effects are not always the same: for example, continuous grazing by ungulates sometimes increases grasshopper richness (Joern 2005), but tends to decrease it in other cases (Gebeyehu & Samways 2003). Such seemingly conflicting outcomes might have resulted from the fact that the different sites were these studies were performed are dominated by different ungulate species, with different ungulate species having contrasting effects on their surroundings (Olff & Ritchie 1998). One ungulate species that is particularly well known for being distinct from other ungulate species, and which has the reputation of being an 'ecosystem engineer' or 'keystone species' in savannah ecosystems, is the white rhino (Owen-Smith 1988; Waldram et al. 2008). This megaherbivore distinguishes itself from other grazing ungulates by having an exceptionally large body size (weighing approximately 1700 kg) and by its unique foraging behaviour. It creates its own mud pools (wallows) and middens in the landscape, and it tends to graze much more intensively around these self-made landscape elements than in other locations in the savannah (Owen-Smith 1988). As a result, it promotes habitat heterogeneity in savannahs (Owen-Smith 1988; Waldram et al. 2008). Probably due to the high quality regrowth of grazed vegetation, white rhinos facilitate smaller ungulate species (mesoherbivores) around wallows (Waldram et al. 2008). As such, one might expect rhinos to exert much larger effects on plant and consequently grasshopper communities than mesoherbivores do.

Apart from the question whether the *magnitude* by which white rhinos and mesoherbivores affect grasshopper communities differs, it is also unknown whether these differently sized ungulates affect grasshopper communities by different *mechanisms*. Grasshoppers respond to various factors of their surroundings: most notably, plant species composition and plant traits (due to species specific diet requirements of grasshoppers) (Joern 2005,2012; Van der Plas et al. 2012) and vegetation structure (due to differences in microclimate and predation risk related with different vegetation structures) (Joern 1982; Kruess & Tscharntke 2002a; Van der Plas et al. 2012). Rhinos and mesoherbivores may differ in which of these properties of their surroundings – plant community composition or vegetation structure – they alter most. While small ungulate species are often considered to be highly selective feeders, 'filtering out' high quality grass species and plant parts from the vegetation, larger sized herbivores are usually considered more as 'bulk feeders' that may affect vegetation structure much more than plant community composition (Hagenah et al. 2009). If so, then one might expect that white rhinos affect grasshopper communities through alterations in vegetation structure, while mesoherbivores affect grasshopper communities through changes in plant community and trait composition: some grasshopper species might get 'outcompeted' by mesoherbivores with similar diets.

To test these ideas, we performed a grazing experiment in a Hluhluwe-iMfolozi, a South African savannah park. Hluhluwe-iMfolozi Park (HiP) harbors many ungulate species, such as buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*) and warthog (*Phacochoerus aethiopicus*). However, in terms of biomass, white rhino is the most abundant. Approximately 1700 white rhinos live in HiP, which thereby culminate 27.3% of the total grazing ungulate biomass (KZN wildlife, 2004 census data) and approximately 8.5% of the white rhino world population (IUCN 2010). Due to this dominance of white rhinos, HiP forms an excellent study system to compare the effects of this megaherbivore with the effects of smaller-sized ungulates on grasshopper communities. Specifically, we asked the following questions: (1) how do community composition and species richness of grasshoppers respond to both rhinos and mesoherbivores; (2) how can grass and grasshopper species associated with different grazing treatments be distinguished from each other in terms of traits; (3) are responses of grasshopper communities to grazing mostly mediated by competitive interactions with grazing ungulates (changes in plant community composition) or by changes in microclimate and/or predation risk (changes in vegetation structure)?

## 2.2 Methods

### 2.2.1 Study site

Our study was performed in Hluhluwe-iMfolozi Park (HiP), an 89 665 ha savannah park in South Africa (28°00'-28°26'S, 31°41'-32°09'E). HiP contains a wide variety of habitat types, ranging from closed upland forests, woodland, bunch grasslands and grazing lawns (Whately & Porter 1983). At a large scale, this mosaic of vegetation types is likely caused by gradients in annual amount of rainfall and fire frequency, while at smaller spatial scales large herbivores have been shown to create and maintain vegetation mosaics (e.g. Cromsigt & Olff 2008).

Annual amount of rainfall in HiP ranges from 450 mm in the south to 750 mm in the north (KZN wildlife, unpublished data). Rain falls mostly between October and April, while in the dry season (May-September) rainfall is usually less than 200 (northern part) or 50 (southern part) mm. Fires are usually ignited by park managers, and fire return intervals in the park range from 2 to 6 years. The park has a high diversity of large herbivore species, with the more common species including elephant (*Loxodonta Africana*), white rhino (*Ceratotherium simum*), black rhino (*Diceros bicornis*), cape buffalo (*Syncerus caffer*), giraffe (*Giraffa camelopardalis*), wildebeest (*Connocheates taurinus*), plains zebra (*Equus burchelli*), kudu (*Tragelaphus strepsiceros*), nyala (*Tragelaphus angasi*), impala (*Aepyceros melampus*) and warthog (*Phacochoerus aethiopicus*).

### 2.2.2 Experimental design

Early 2000, the experiment was set up (Bond and Olff. unpubl. data). Eight sites, differing in geology (Stock et al. 2010), were chosen (Fig. S2A) throughout the park to investigate the effects of megaherbivores and mesoherbivores on plant and animal communities. In order to do so, at each site, two 40 x 40 m plots were fenced: the first plot (hereafter: 'mesoherbivore grazing plot') was fenced with a single cable at a height of 50 cm, excluding white rhino, but still allowing mesoherbivores and insects to enter. The second plot (hereafter: 'microherbivore grazing plot') was fenced to a height of 2.5 m to exclude all ungulates larger than scrub hares (*Lepus saxatilas*), thus only allowing 'microherbivorous' rodents and arthropods. Additionally, an adjacent, 40 x 40 meter control plot (hereafter: 'megaherbivore grazing plot' was selected where no herbivores were excluded: both white rhinos, mesoherbivores and 'microherbivores' could graze here. After the experiment was set up, all sites were burnt biannually (2002, 2004, 2006 and 2008, in August or September). The two closest sites were 506 meters apart, but most other sites were much further away from each other, up to ~30 km (Fig. S2A). In January – May 2010, ten years after the experiment was set up, we characterized grass and grasshopper community composition.

### 2.2.3 Grass community data

Grass species composition of each plot in each site was characterized at four different scales. In each plot, a grid spanning in total 20 x 36 meter was laid out, with individual grid cells measuring 2 x 2 meter. At each intersection point (180 points in total), the most dominant grass species was identified using Van Oudtshoorn (2002). Additionally, using a disc pasture meter (diameter: 46 cm, mass: 460 g), the vegetation height was measured at each intersection point.

To characterize the grass density at finer resolutions, within the 20 x 36 meter grid, at the corners smaller subgrids were defined: four 18 x 10 meter subgrids (45 intersection points), four 10 x 6 meter subgrids (15 intersection points) and four 6 x 4 meter subgrids (6 intersection points). Also in these subplots, the number of unique dominant grass species was counted. Additionally, at all these scales, heterogeneity was calculated as the coefficient of variation in vegetation height.

### 2.2.4 Grasshopper community data

At each site and each plot, grasshoppers were sampled using 2 different methods: by standardized sweep netting along transects and by target catching of grasshoppers during a 10 minute period. We walked three 40 m transects within each plot, spaced 5 meters from each other and within the part of the plot where the grass communities were described. We walked each transect up and down while sweeping with a net in the vegetation at a height between 0 and 50 cm above the ground. Immediately

after finishing walking a transect, the whole content of the sweepnet was emptied into a sealable plastic bag. After catching grasshoppers along the transects, we caught grasshoppers for 10 minutes using a 'target catching' technique: we tried to catch all grasshoppers that were located visually or by ear. We also noted grasshopper species that were observed during those 10 minutes, but that we failed to catch. All plots within a site were sampled on the same day and only on days with fair weather, in order to prevent weather conditions from biasing the outcome of our results. At the end of each day in the field, samples were frozen at -18°C for preservation. Each plot was visited on two dates between mid January and mid February (South African summer) and again two dates between mid March and mid May (South African autumn). We thus collected sixteen grasshopper samples in each plot and 384 samples in total. After the field period, all samples were brought to the University of Groningen (Netherlands) and adult grasshoppers were identified using Dirsh (1965) and Johnsen (1982,1990).

### 2.2.5 Grass and grasshopper trait data

We measured four different traits: specific leaf area (SLA), individual leaf area (LA), canopy height and leaf nitrogen (N) content. Grass traits were measured from individuals of each species, taken at each site: preferably in the megaherbivore grazing plot or surrounding 'control' vegetation, unless it only occurred in the mesoherbivore or microherbivore grazing plot. LA is the surface area of an individual fresh leaf and high LA tends to increase transpiration and therefore lower drought tolerance (Westoby et al. 2002). SLA is LA divided by its dry weight. Species with high SLA usually have high growth rate, but low leaf life span (Reich et al. 1997; Weiher et al. 1999; Westoby et al. 2002). To measure SLA and LA, we collected between 10 and 30 leaves from different individuals of each species and stored them in moist tissue paper within a cool box to keep them fresh. At the end of each day, leaves were brought to the Hluhluwe Research Station, where they were photographed together with a black reference square of 2 x 2 cm. LA (in $cm^2$) was then calculated using the ImageJ software (Abramoff et al. 2004). After photographing the leaves, they were dried in an oven at 50°C for at least 48 hours, after which dry weight was measured. SLA (in $cm^2\ g^{-1}$) was then calculated by dividing LA by leaf dry weight. Plant canopy height (in cm), was measured in the field for at least 5 individuals of each species, by measuring the height from the base of the tallest leaf. Plant canopy height is related to the ability to compete for light (Weiher et al. 1999). To measure leaf N content, we collected fresh, green leaves of each species in each plot, at least at five different locations. These leaves were put in a paper bag and brought to Hluhluwe Research Station, where they were dried in an oven at 50°C for at least 48 hours. Samples were then brought to the University of Groningen (Netherlands) and ground with a ball mill. For approximately half of the samples, we randomly selected 2 x 5 mg of the leaf material and measured N concentrations in duplo using a Carlo-Erba NA 1500 element analyzer (Carlo-Erba, Milan, Italy). We then estimated N content of the remaining samples using a Near InfraRed (NIR) spectrophotometer (Bruker MPA NIR), with a calibration line constructed using OPUS 7.0 software and based on the leaf N concentrations measured in this study and measurements done for other savannah grass studies. Plants with high leaf N content usually have a high growth rate (Reich et al. 1997) and are generally attractive food for herbivorous insects (Behmer 2009).

We measured two different grasshopper traits. Body length and wing length (both in mm) were measured at the University of Groningen (Netherlands) of each adult grasshopper individual collected. Body size is related to food choice (Belovsky 1997) and predation risk (Belovsky et al. 1990), while wing length is related to dispersal (Harrison 1980).

After trait measurements, we calculated average trait values for each grass and grasshopper species. These species trait values (STV) were then used to calculate abundance-weighted trait values in communities (Community Abundance-Weighted Trait Average or CAWTA): $CAWTA = \sum_{i=1}^{S} a_i\ STV_i$, in which S is the species richness in the given community, $a_i$ is the abundance of the i-th species and $STV_i$ is the STV of the i-th species. For grasses, CAWTA values were only calculated at the largest plot (20 x 36 meter) scale.

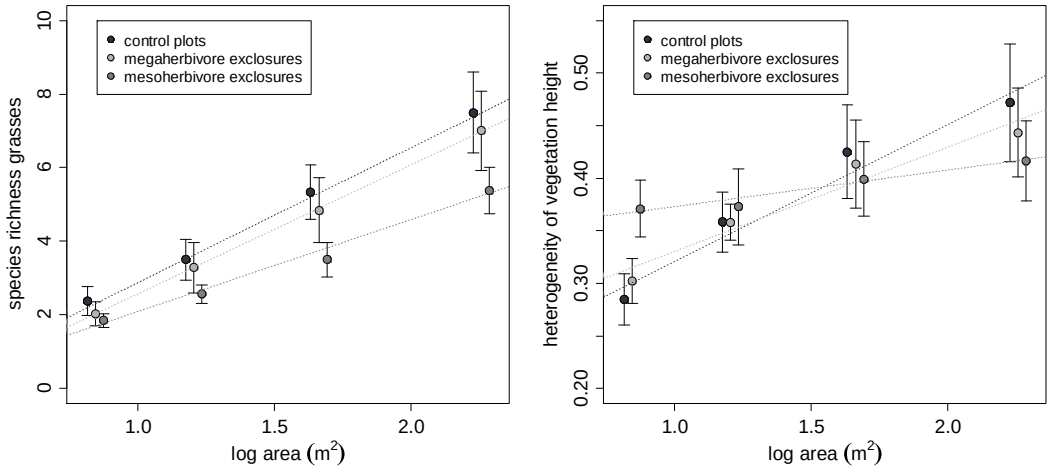


**Figure 2.1.** Plots showing the relationships between (a) species richness of grasses and (log) area, in both ungrazed (hare fence) and intensively grazed (control, rhino fence) plots and (b) between heterogeneity and (log) area in both control, rhino fence and hare fence plots. While species richness and heterogeneity increases with area in both intensively grazed and ungrazed plots, this increase is higher for intensively grazed plots. A similar pattern is found for heterogeneity: at small scales, this is highest in hare fence plots and lowest in control fence plots, while this pattern is reversed at higher scales.

### 2.2.6 Identifying the effects of grazing on grass and grasshopper communities

Using general linear mixed models (LMMs), with full models containing treatment, rainfall and their interaction as fixed effects and site as a random effect, we investigated how grazing and rainfall affect vegetation height and CAWTA values. Also, using an LMM with site as a random effect, we investigated how grass richness and heterogeneity were explained by log area and grazing intensity. We checked whether assumptions of normality were met. Using a maximum likelihood approach, we selected a model with lowest AIC value. LMMs were done with the lme function (nlme package, Pinheiro et al. 2011) in R-2.13.1 (R Development Core team 2011). We used generalized linear mixed models (GLMMs) with full models containing treatment, rainfall and their interaction as fixed effects and site as a random effect to investigate how grazing and rainfall affect grasshopper species richness and abundance. We checked whether GLMM residuals conformed to the assumed poisson distribution. Using a maximum likelihood approach, we selected a GLMM model with lowest AIC value. These tests were done with the lmer function (lme4 package, Bates et al. 2011) in R-2.13.1 (R Development Core team 2011). When selected GLMM or LMM contained a significant grazing factor, we performed a post-hoc Tukey-HSD test using the 'glht' function from the multcomp package (Hothorn et al. 2008) in R to investigate which grazing regimes differed from each other. Differences in community composition of grasses and grasshoppers between treatments were visualized using Non-Metric Dimensional Scaling (NMDS) with the nmds function (library: ecodist, Goslee and Urban 2007) in R. We then used MANOVAs to investigate whether Bray-Curtis distances in community composition could be explained by grazing treatment and rainfall, while taking site differences into account as a random factor. This was done using the veg.dist and adonis (library: vegan, Oksanen et al. 2010) functions from R. We used Dufrene and Legendre's (1997) analysis to investigate which species were significant habitat indicator species, using the indval function (library: labsdv, Roberts 2010). With Multiple Regression Analyses, we additionally explored how grasshopper community variables were related to grasshopper community variables.

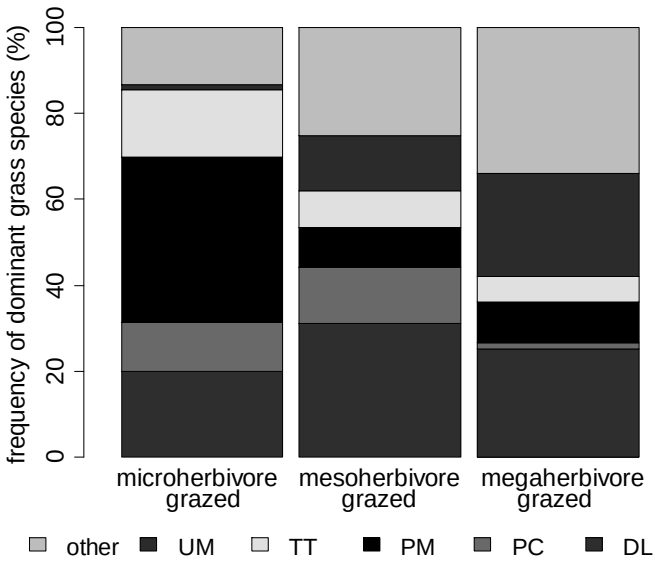


**Figure 2.2**. Frequency of the dominant grass species in plots of different grazing treatments: microherbivore grazing plots (left), mesoherbivore grazing plots (middle) and megaherbivore grazing plots (right). *Panicum maximum* clearly responds negatively to mesoherbivore grazing. Abbreviations: UM = *Urochloa mosambicensis*, TT = *Themeda trianda*, PM = *Panicum maximum*, PC = *Panicum coloratum*, DL = *Digitaria longiflora* and other = other (less commonly observed) grass species.

# 2.3 Results

## 2.3.1 Vegetation structure and grass and grasshopper community composition

Grazing significantly reduced vegetation height, which was taller in microherbivore grazing plots than in megaherbivore and mesoherbivore grazing plots (Table 2.1). There was an interaction effect between scale and treatment on heterogeneity of vegetation height (Table 2.1): at small scales, heterogeneity was lowest in control plots and highest in hare fence plots, while this pattern was reversed at larger scales (Fig. 2.1). There was additionally an interaction effect between scale and treatment on grass species richness: grazing increased grass richness, but much more so at larger scales (Table 2.1, Fig. 2.1). One dominant grass species was a significant habitat indicator species: *Panicum maximum* was associated with microherbivore grazing plots (Fig. 2.2). When abundance data of megaherbivore grazing and mesoherbivore grazing plots were pooled ('high intensity grazing plots'), two dominant grass indicator species could be identified: *Panicum maximum* for low intensity grazing (i.e. microherbivore grazing) plots and *Sporobolus nitens* for high intensity grazing plots. A multivariate analysis confirmed the idea that community composition of grasses differed significantly between the grazing treatments and also responded to rainfall (MANOVA, $P = 0.032$, Fig. 2.3).

Grasshopper species richness and abundance were not significantly affected by ungulate grazing (Table 2.1). However, grasshopper species richness correlated marginally significantly with vegetation height (Multiple Regression Analysis: $F_{1,22} = 4.205$, $P = 0.052$, $R^2 = 0.160$), but not with species richness of grasses or heterogeneity in vegetation height, irrespective of scale. Grasshopper abundance did not correlate with vegetation height, heterogeneity or grass species richness, irrespective of scale (Multiple Regression Analysis). Three habitat indicator grasshopper species were identified: *Cataloipus zuluensis*, *Tylotropius didymus* and *Catantops melanostrictus* were associated with hare fence plots. When comparing high (megaherbivore and mesoherbivore grazing) with low intensity (microherbivore) grazing plots, six grasshopper indicator species could be identified:

*Cataloipus zuluensis*, *Catantops melanostrictus*, *Tylotropius didymus* and *Leptacris monteiroi* for low intensity grazing plots and *Acrotylus furcifer* and *Tmetonota rugosa* for high intensity grazing plots (Fig. 2.3). Grasshopper community composition differed significantly between the three treatments and rainfall (Fig. 2.3, MANOVA, $P = < 0.001$). Further analyses showed that these responses to grazing were regulated by changes in grass community composition and grass traits: $NMDS1_{grasshopper}$ increased with NMDS1 and NMDS2 of grasses and decreased with the CAWTA of SLA (Multiple Regression: $F_{3,20} = 3.19$, $P = 0.046$, $R^2 = 0.324$), $NMDS2_{grasshopper}$ increased with $NMDS3_{grasses}$ (Fig. 2.3,2.4; Multiple Regression: $F_{1,22} = 24.46$, $P < 0.001$, $R^2 = 0.527$) and $NMDS3_{grasshopper}$ decreased with NMDS 2 and NMDS3 of grasses and increased with the CAWTA of grass height and leaf nitrogen content (Fig. 2.4; Multiple Regression $F_{4,19} = 11.10$, $P < 0.001$, $R^2 = 0.700$).

### 2.3.2 Grass and grasshopper traits

Mesoherbivore grazing significantly reduced the CAWTA of LA: LA was lower in megaherbivore and mesoherbivore grazing plots than in microherbivore grazing plots (Table 2.1). CAWTAs of all other grass traits were not affected by grazing, although there was a trend for lower canopy height values in megaherbivore and mesoherbivore grazing plots than in microherbivore grazing plots (Table 2.1). For grasshoppers, mesoherbivore grazing reduced the CAWTA of body length, with body length being smaller in megaherbivore and mesoherbivore grazing plots than microherbivore grazing plots (Table 2.1). Residual wing length was not affected by grazing (Table 2.1). Grasshopper body length CAWTA appeared to correlate strongly with vegetation height ($R^2 = 0.704$), but not with heterogeneity or CAWTA values of grass traits (Fig. 2.4, Multiple Regression Analysis, $F_{1,22} = 52.31$, $P < 0.001$). CAWTA of wing length did not correlate with vegetation structure or CAWTA values of grasses, but it did correlate positively with rainfall (Multiple Regression Analysis, $F_{1,22} = 5.148$, $P = 0.033$, $R^2 = 0.190$).

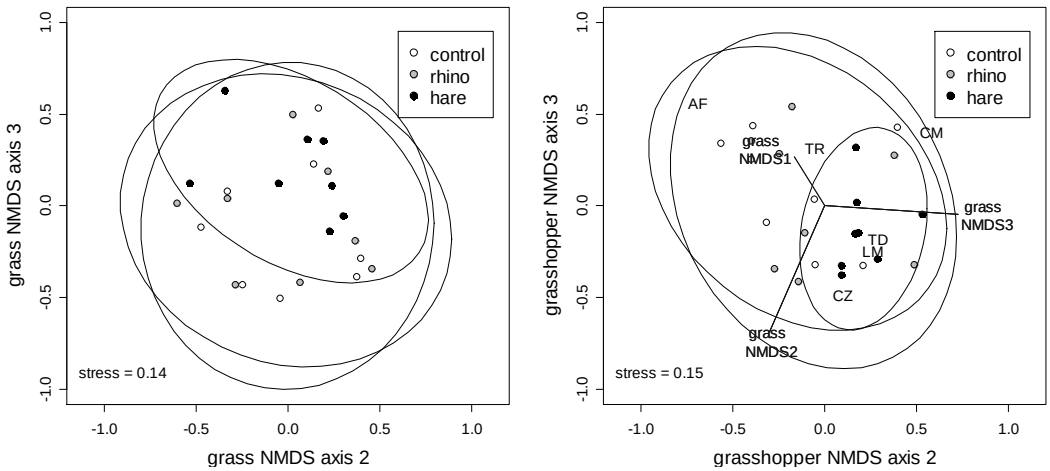


**Figure 2.3.** Biplots of NMDS axes of grass (top) and grasshopper (bottom) communities. In the right plot, arrows indicating how NMDS axes of grasses correlate with those of grasses are shown. Control plots are shown in white, rhino fence plots in grey and hare fence plots in black. Ellipses around equal-coloured dots represent the two-dimensional confidence intervals (± 2SD) of average NMDS values of different grazing treatments. Significant habitat indicator species are shown in NMDS space with their abbreviations: for grasses: SN, *Sporobolus nitens* (high intensity grazing indicator species); PM, *Panicum maximum*; TT, *Themeda triandra* (both low intensity grazing indicator species); for grasshoppers: AF, *Acrotylus furcifer*; TR, *Tmetonota rugosa* (both high intensity grazing indicator species); CZ, *Cataloipus zuluensis*; CM, *Catantops melanostrictus*; TD, *Tylotropius didymus*; LM, *Leptacris monteiroi* (all low intensity grazing species).

**Table 2.1.** Best models resulting from a general(ized) linerar model selection (ML procedure) in which the effects of grazing and rainfall on grass and grasshopper diversity, community composition and trait values were investigated. Response variables are given in the left column, average values and SEMs for each grazing treatment are given in the middle three columns and *F* (or $\chi^2$) and *P* values of significant models in the right column

| Response variable | Control | Rhino | Hare | Best model |
|---|---|---|---|---|
| **General patterns** | | | | |
| Vegetation height (cm) | 14.73 ± 4.754[a] | 17.48 ± 5.325[a] | 23.02 ± 3.714[b] | Grazing (F = 6.817, P = 0.009) + rainfall (F = 6.917, P = 0.045) |
| Height heterogeneity | 0.385 ± 0.023[a] | 0.379 ± 0.018[a] | 0.390 ± 0.017[a] | Grazing(F = 0.240, P = 0.787) + rainfall (F = 0.664, P = 0.418) + log area (F = 57.60, P < 0.001) + log area x grazing (F = 5.862, P = 0.004) |
| Grass richness | 4.680 ± 0.498[a] | 4.289 ± 0.502[ab] | 3.320 ± 0.310[b] | Grazing (F = 12.74, P < 0.001) + log area (F = 226.47, P < 0.001) + log area x intensity (F = 3.01, P = 0.055) |
| Grasshopper richness | 11.25 ± 1.411[a] | 11.13 ± 0.833[a] | 12.75 ± 0.818[a] | NS |
| Grasshopper abundance | 57.00 ± 17.624[a] | 55.13 ± 17.326[a] | 49.25 ± 6.700[a] | NS |
| **Grass traits** | | | | |
| LA ($cm^2$) | 6.658 ± 1.002[a] | 6.399 ± 1.104[a] | 9.249 ± 1.145[b] | Grazing (F = 5.435, P = 0.018) |
| SLA ($cm^2g^{-1}$) | 249.1 ± 11.36[a] | 244.8 ± 10.97[a] | 256.6 ± 14.64[a] | NS |
| Leaf N content (%) | 2.120 ± 0.080[a] | 2.195 ± 0.082[a] | 2.282 ± 0.131[a] | NS |
| Canopy height (cm) | 36.79 ± 4.884[a] | 34.40 ± 6.017[a] | 44.45 ± 3.890[a] | Grazing (F = 1.976, P = 0.178) + rainfall (F = 1.973, P = 0.184) |
| **Grasshopper traits** | | | | |
| Body length (cm) | 21.975 ± 1.008[a] | 22.064 ± 1.281[a] | 25.544 ± 0.912[b] | Grazing (F = 14.287, P < 0.001) + rainfall (F = 3.943, P = 0.069) |
| Resid. wing length (cm) | 0.297 ± 0.383[a] | -0.191 ± 0.331[a] | -0.105 ± 0.281[a] | NS |

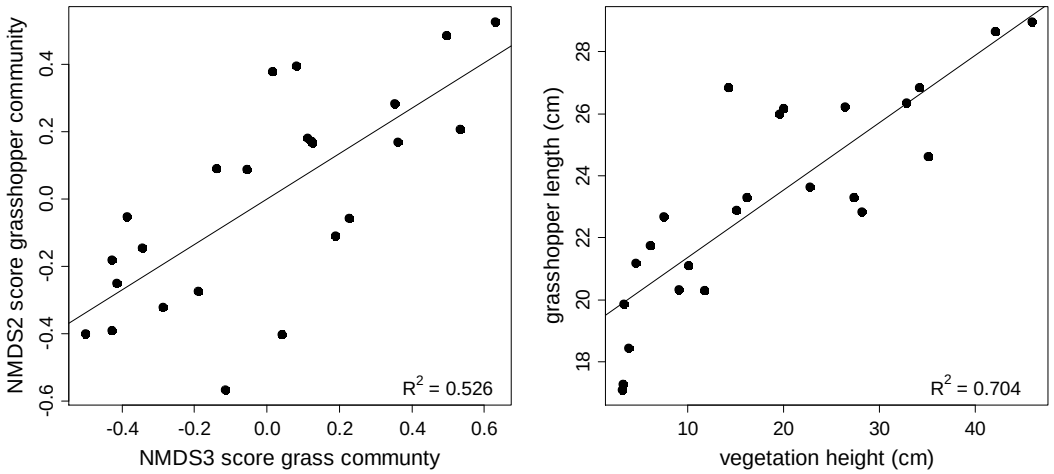


**Figure 2.4.** The relationships between grass (NMDS axis 3) and grasshopper (NMDS axis 2) community composition (top) and between vegetation height and the community-weighted average (CAWTA) of grasshopper length (bottom).

## 2.4 Discussion

In this study, we investigated, using a long-term exclosure experiment, how grazing by megaherbivores and mesoherbivores affects grasshopper communities. Ten years after these exclosures were set-up, it was clear that grazers consume a large portion of the grass biomass: in mesoherbivore grazing plots, the grass vegetation was 24.1% shorter than in microherbivore grazing fence plots. Mesoherbivores probably ingest even more than this percentage of the grass biomass, but due to fast regrowth after defoliation (McNaughton 1992) vegetation biomass measures in the field tend to underestimate biomass consumption rates. Surprisingly, there were no significant differences in vegetation height between megaherivore (white rhino) and mesoherbivore grazing plots. White rhinos are very abundant in HiP, accounting for approximately 27.3% of the total grazing ungulate biomass (KZN wildlife census 2004, unpublished data) and are suggested to be keystone species (Owen-Smith 1988) and ecosystem-engineers, that facilitate for mesoherbivores (Waldram et al. 2008). Therefore, we expected them to have a large effect on the vegetation. Instead, rhinos did, unlike mesoherbivores, neither (significantly) reduce vegetation height, nor any other aspect of grass communities, and consequently not grasshopper communities either. Other studies have suggested that while rhinos may facilitate mesoherbivores in the rainy season, they may compete with them in drier parts of the year (Arsenault and Owen-Smith 2002, 2011). If these competitive effects on mesoherbivores are stronger than facilitative effects, increased mesoherbivore grazing might compensate for the exclusion of white rhino. In line with this interpretation, another study performed at the same exclosure sites showed that dung counts of grazing ungulates are equally high in megaherbivore grazing plots as in mesoherbivore grazing plots (Staver et al. 2009). However, our results are seemingly conflicting with results from Waldram et al. (2008), who found that rhinos reduce vegetation height and thereby facilitate for (rather than compete with) other herbivore species. Perhaps, our results are different because our study, unlike the study of Waldram et al. (2008), was not specifically performed around areas of most intense use by rhinos (wallows). Nonetheless, while grazers clearly affect the vegetation in HiP, effects of white rhinos alone were almost negligible in our study.

Unlike white rhinos, mesoherbivores had strong effects on both vegetation height and plant community composition, with important consequences for grasshopper communities. Most conspicuously, excluding mesoherbivores transformed the grassland into a dense vegetation dominated by the large-leaved *Panicum maximum* away from a less dense vegetation were small-

leaved lawn grass species, such as *Sporobolus nitens* and *Urochloa mosambicensis*, were more dominant (Fig. 2.2). Possibly, the nutritious *Panicum maximum* cannot persist the continuous grazing by mesoherbivores (Kleynhans et al. 2011), while the small leaved grass species associated with mesoherbivore grazed plots are better able to persist the trampling-induced drought (Belsky 1986), due to decreased evaporation associated with small leaf size (Westoby et al. 2002). The shifts in vegetation characteristics were associated with shifts in grasshopper communities: plots where mesoherbivores were excluded mostly contained large grasshopper species of various subfamilies, while plots with mesoherbivore (and megaherbivore) grazing were dominated by much smaller species, often from the subfamily Oedipodinae. This raises the question by which mechanism mesoherbivores alter grasshopper communities. Direct effects seem unlikely, since grasshoppers are mobile insects that should not get easily disturbed by ungulates. Previous studies have shown that usually, vegetation structure and plant community composition are more important determinants for communities of grasshopper or other insects (Joern 1982; Kruess & Tscharntke 2002a; Joern 2005; Joern et al. 2012; van der Plas et al. 2012) and therefore good candidates for explaining responses of grasshopper communities to grazing.

We found that grasshopper body size was highly correlated with vegetation height. This suggests that through reducing the vegetation height, mesoherbivores favor small grasshopper species over larger ones, leading to observed shifts in communities. Vegetation height is a rough measure for the total amount of plant biomass available for herbivorous insects, so it could be that sites grazed by mesoherbivores offer insufficient quantities of food for large grasshopper species. Another possibility is that large grasshopper species cannot live in low vegetation, because in these sites, they cannot find coverage for bird predators, which often feed on larger species (Belovsky et al. 1990). A third possible reason why smaller grasshoppers live in short vegetation, is because of interspecific differences in microclimate requirements. Grasshoppers often respond to differences in temperature (Ritchie 2000), and larger insect species might be better able to regulate body temperature than smaller insect species. It might thus be that smaller grasshopper species need hotter microclimates, found in areas with low vegetation, to develop or reproduce.

While vegetation height thus appeared to be partially explaining the response of grasshopper communities to grazing by mesoherbivores, the species composition (but not richness) of grass communities appeared to be similarly important. This suggests that even though grasshoppers are generally considered generalist feeders, not confined to only one food plant, they nevertheless do have preferences for certain plant species. Possibly this could be because of plant species differences in nutrient levels. A nutrient that is usually considered to be a very important determinant in explaining diets of herbivores, is nitrogen (Behmer 2009). We therefore expected that grass community composition would drive grasshopper communities, this would be because grass species of different communities differ in nitrogen concentrations. However, average leaf nitrogen values of dominant grass species did not respond to grazing and additionally, these community-average values of leaf nitrogen content did neither correlate with grasshopper body size, nor with ordination axes of grasshopper communities. The fact that community-average values of leaf nitrogen did not respond to grazing was somewhat surprising, since both other studies (McNaughton 1985) and this study show that grazing promotes the dominance of nitrogen-rich, clonally growing lawn grass species. However, grazing also led to a reduction of the very nitrogen-rich *Panicum maximum* (Fig. 2.2). Although it should be noted that we only focused on interspecific (rather than intraspecific) variation in nitrogen levels and that it might be that grazing did lead to the promotion of nitrogen rich regrowth within species (McNaughton 1985,1992), our study does not confirm the idea that grasshoppers prefer certain grass communities due to associated high nitrogen levels. Other studies have shown that other nutrients than nitrogen, such as phosphorous, can sometimes be more important in explaining grasshopper population densities or community assembly (Joern 2012), although more work is needed to conclude whether this also holds in our study system. In any case, we showed that grasshoppers do not only respond to grazing by mesoherbivores through changes in vegetation height, but additionally through changes in grass community composition.

Our study has important implications for conservation management. Firstly, the finding that the grazing by mesoherbivores leads to communities containing *different, but not more or fewer* grasshopper species, suggests that for conserving as many grasshopper species as possible, maintaining both grazed and ungrazed vegetation is important. Our study site HiP is, like many other African savannah reserves, characterized by very high densities of ungulates (Prins & Olff 1998; Cromsigt et al. 2009). However, that is not to say that ungulates are everywhere: in the wetter sites of HiP, but also in wet areas of other savannah systems, ungulate densities are usually much lower due to the low quality of dominant grasses (Olff et al. 2002), while also in drier savannah sites grazers sometimes leave some patches ungrazed. This might mean that even with the currently high densities of ungulates, grasshopper species preferring high, ungrazed vegetation are still present in most savannahs, albeit in relatively low numbers. Secondly, the finding that unlike mesoherbivores, megaherbivores did not affect grasshopper communities in our study, suggests that at least for most savannah area, reintroductions or local extinctions of rhinos will not affect grasshopper communities. However, this should not be interpreted as a case against the wildlife conservation of white rhinos. Firstly, numbers of white rhinos went down in recent years due to a dramatic increase in illegal poaching (Thomas 2010), making the conservation of white rhino already an important goal by itself. Additionally, other studies have shown that around areas of intense habitat use by rhinos, such as wallows, rhinos do have strong effects on their abiotic surroundings, the vegetation and other ungulates (Waldram et al. 2008), and it is not unlikely that at those places also insects such as grasshoppers are affected by rhinos. Nevertheless, at most 'random' or 'matrix' savannah sites, conservation management of white rhinos is, unlike the management of mesoherbivores, not expected to have strong effects on grasshopper communities.

## 2.5 Acknowledgements

We thank William Bond and the ZLTP team for setting up the exclosures investigated in this study. Additionally, we thank the SABRE and ZLTP team for maintaining the exclosures over the years. We are also grateful for Maarten Schrama, Nelly van Eck, Julia Wakeling and the SABRE team for the coordination of, and helping with, the data collection. Thanks must also go to Roel van Klink, Cleo Graf and Maarten Schrama, who provided useful comments about earlier versions of this manuscript. Finally, we want to thank KZN wildlife for granting us the permission for performing this study.

# Chapter 3: Effects of large herbivores on grassland arthropod diversity

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## 3.1 Abstract

Both arthropods and large grazing herbivores are important components and drivers of biodiversity in grassland ecosystems, but a synthesis of how arthropod diversity is affected by large herbivores has been missing. To fill this gap we conducted a search of published literature, which yielded 143 studies on this topic of which 24 could be used to quantitatively assess the effects of grazing on plant and arthropod diversity. This quantitative assessment shows no overall significant effect of increasing grazing intensity on plant diversity, while arthropod diversity is generally negatively affected by large herbivores. To understand these often negative effects, we explore the main routes through which large herbivores affect arthropod communities: direct effects, changes in vegetation structure, changes in plant community composition, changes in soil conditions, and cascading effects within the arthropod interaction web.

We identify three main factors determining the effects of large herbivores on arthropod diversity: 1) unintentional predation and disturbance increase with grazing, 2) total resource abundance for arthropods (biomass) decreases with grazing, and 3) plant diversity and heterogeneity in vegetation structure and abiotic conditions can increase at intermediate intensity of grazing, but decline when grazing intensity is too high.

We conclude that large herbivores can only increase arthropod diversity if they cause an increase in (a)biotic heterogeneity, and if this increase is large enough to compensate for the loss of total resource abundance and the increased mortality rate. This is expected to occur only at low abundances of herbivores.

As we demonstrate that arthropod diversity is often more negatively affected by grazing than plant diversity, we strongly recommend to consider the specific requirements of arthropods when applying grazing management and to include arthropods in monitoring schemes. Conservation strategies aiming at maximizing heterogeneity, such as the regulation of low densities of herbivores (through human interventions or top-down control), maintenance of different types of management in close proximity, or rotational grazing regimes, are most likely to conserve arthropod diversity.

## 3.2 Introduction

Large grazing herbivores exert major influences on their habitat and are abundant and important in all grassland ecosystems (Hobbs 1996; Olff et al. 2002). Populations and communities of large herbivores have been under human influence for millennia, with humans causing extinctions (Owensmith 1989; Lorenzen et al. 2011; Rule et al. 2012) and changes in abundances (Owensmith 1989). Additionally, ever since the first goats and sheep were domesticated over 11000 years ago (Zeder, 2008) agricultural livestock practices have been intensified, culminating in the year 2000 into 26 % of the terrestrial biome being used for livestock production as pasture or fodder crops (FAO 2008). This may pose a threat to biodiversity through overgrazing (e.g. Smith 1940), and can lead to habitat loss and fragmentation (e.g. Kruess & Tscharntke 1994; Fahrig 2003). Conversely, in many semi-natural types of grassland, especially in Europe, the maintenance or reintroduction of large herbivores is a widely applied management tool, aiming to preserve an open, species-rich landscape (WallisDeVries 1998; Ostermann 1998). In these systems, livestock is thought to replace ecological functions of now-extinct native herbivores as aurox and tarpan (Bakker et al. 2004). Due to the enormous impact of grazing on a global scale and especially because of recent changes in grazing

regimes in many areas, it is imperative to understand the influence of large, grazing herbivores on the biodiversity of various plant and animal groups.
Effects of grazing on plant diversity are variable, with literature supporting both positive and negative effects (Milchunas et al. 1988; Olff & Ritchie 1998). Reported effects on arthropod diversity are equally diverse, with studies reporting negative (e.g. Kruess & Tscharntke 2002a,b; Pöyry et al. 2004), positive (Joern 2005; Woodcock & Pywell 2009), or neutral (Bestelmeyer & Wiens 2001; Hofmann & Mason, 2006) effects of large herbivores. Intuitively, a strong positive relation between the diversity of resources (plants) and consumers (arthropods) would be expected (Murdoch et al. 1972; Tilman 1986), but evidence is mounting that the response of arthropod diversity to grazing deviates from that of plant diversity (Kruess & Tscharntke 2002a; Pöyry et al. 2006; Zhu et al. 2012). For plants, a number of mechanisms underlying the effects of grazing on diversity have been identified, and general frameworks bringing these mechanisms together have been proposed (Milchunas et al. 1988; Olff & Ritchie, 1998). Such a framework is largely missing for understanding effects of herbivores on arthropod diversity, despite the fact that arthropods constitute the most species-rich animal group on earth, are responsible for myriad ecosystem services (Prather et al. 2012) and take a central place in all terrestrial food-webs (Seastedt & Crossley 1984).
In this review we fully explore the patterns and processes of grassland arthropod responses to large herbivores. First, we present an overview of published literature in terms of taxonomic, geographic and experimental focus in published research, and perform a quantitative review in which we compare the responses of arthropod and plant diversity to grazing. Next, we classify the mechanisms through which large herbivores affect arthropod diversity. The resulting framework includes both direct effects (such as disturbance, incidental predation) and indirect effects (through modifications of soil and vegetation properties) of large herbivores on arthropod communities. Finally, we will synthesize these effects, discuss the implications for conservation of arthropod diversity and identify remaining open questions.
We focus this review on the effects of large herbivores on *aboveground* arthropod communities in *open landscapes* and on *ecological* time scales. Obviously, large herbivores also affect belowground communities (as reviewed by Bardgett & Wardle (2003)), play a role in forested landscapes (reviewed by Suominen & Danell (2006)) and have co-evolutionary relations with grassland plants (McNaughton 1984; Milchunas et al. 1988) and arthropods (e.g. Siegfried 1990). Given these earlier syntheses, these habitats, ecosystem compartments and evolutionary time scales fall outside the scope of this review. Other potentially important drivers of the diversity of grassland arthropods, such as burning and hay-making have been included in reviews by Morris (2000), Littlewood et al. (2013) and Joern & Laws (2013), and are, therefore, also not considered here. Large-scale patterns and processes, such as landscape characteristics and meta-community dynamics have recently been reviewed and synthesized by Tscharntke et al. (2012).

## 3.3 Quantitative response of arthropod diversity to grazing

### 3.3.1 Methods

We performed a systematic search (Pullin & Stewart 2006) for papers on effects of grazing by large herbivores on arthropod species richness, comparing different grazing intensities, species or breeds, or which compared grazing to other forms of conservation management such as burning, haying or abandonment. Only studies meeting the following three criteria were assessed: 1) Published or in press in international, peer-reviewed scientific journals in ISI Web of Science, accessible to the University of Groningen; 2) Performed in (semi)-natural grass- or heathland ecosystems; 3) With arthropods identified to species level. Studies in which grazing effects were potentially confounded with other variables (as soils or climate) were omitted. We initially used cross-referencing to get an overview of the groups of arthropods commonly assessed, and finally performed searches on each of these groups, as well using general search terms such as “insects”, “arthropods” and “invertebrates” in combination with “graz*” in ISI Web of Science. We documented treatment levels, duration, and identity and number of assessed taxonomic groups (class, order or family).

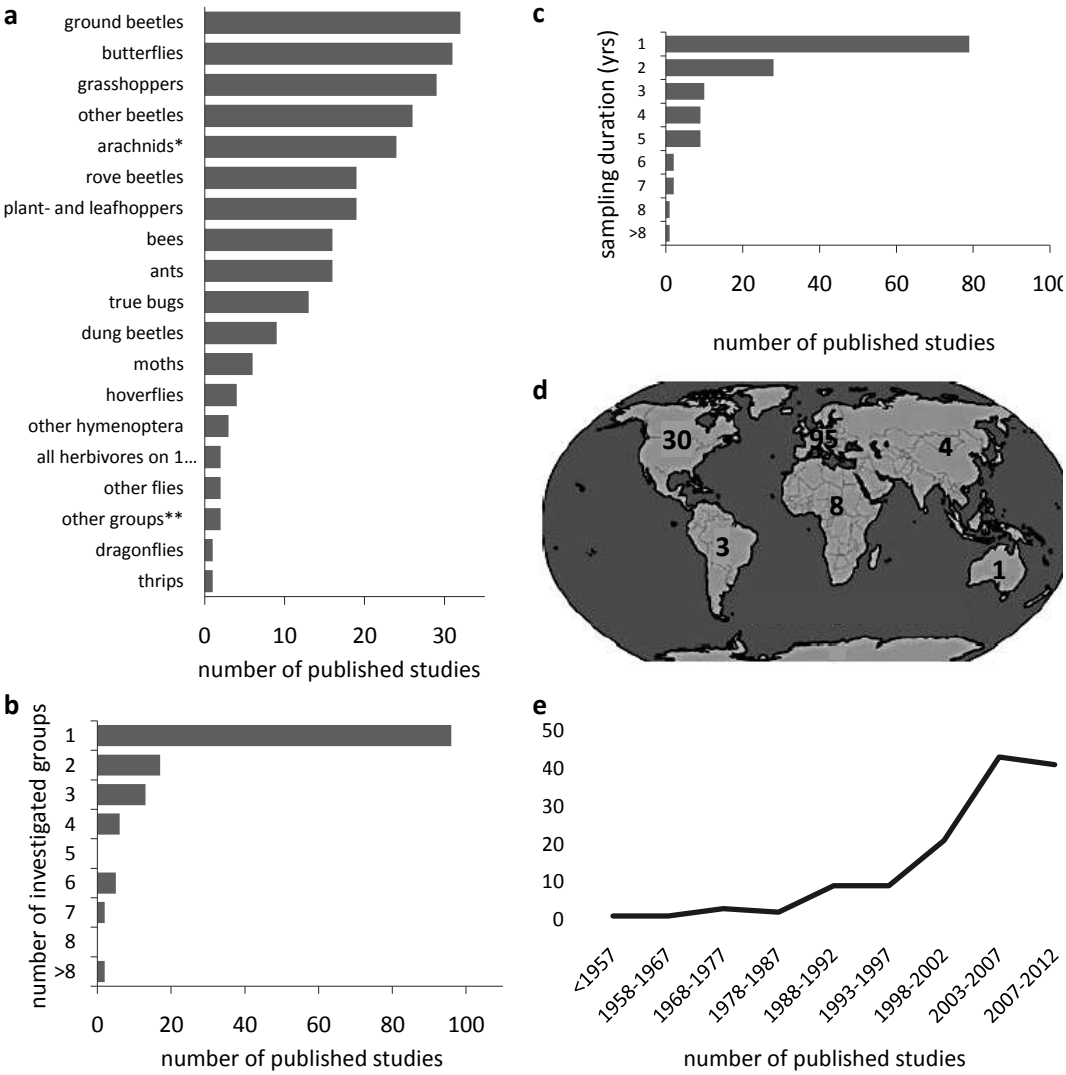


**Figure 3.1.** Research focus of 145 published studies assessing effects of large herbivores on arthropod diversity, conducted in open landscapes (grass- or heathlands), with arthropods identified to species level: a) studied taxa, b) taxonomic spread (number of investigated taxa), c) duration of sampling, d) geographic location, and e) year of publication. We documented the identity of the most commonly assessed taxonomic groups (usually to order level, but sometimes to family or class level). *Arachnids: spiders, harvestmen, pseudoscorpions; **Other groups: Mantodea, Phasmatodea, Neuroptera, Dermaptera etc.

For our quantitative assessment of arthropod diversity response to grazing, we only assessed studies that reported how both arthropod and plant diversity responded to different grazing intensities (including no grazing). In three cases effects on plant diversity were extracted from other publications about the same experiment, and in three cases effects on plant or arthropod richness were directly obtained from the authors. For studies where plant or arthropod richness responses to grazing were only reported in graphs, we used the ImageJ software (Abramoff et al. 2004) to extract an accurate estimate of richness values.

As the response variable for the analyses, we used untransformed response ratios of the change in richness with an increase in grazing intensity ($\frac{r_2 - r_1}{r_1}$, where $r_1$ = richness at lower grazing intensity and $r_2$ = richness at higher grazing intensity), because these better approximated a normal distribution than log-transformed response ratios (Hedges et al. 1999; see also Milchunas & Lauenroth 1993; Wardle et al. 2001). When responses of multiple arthropod taxa were reported (10 studies), we used proportional change averaged over all taxa, so that changes in species-poor taxa (e.g. butterflies) would not be overshadowed by changes in species-rich taxa (e.g. beetles). Therefore, only one data point per comparison between two grazing levels was included for each study. When more than two grazing intensities were reported in a study, all pairwise comparisons were included as separate data points, as were multiple sites per study (whenever reported separately).

Because the data of both plants and arthropods approximated a normal distribution, we used student's t-tests to analyze whether plant or arthropod diversity responded to grazing management and paired t-tests to test whether changes in plant and arthropod diversity differed. To analyze the factors determining arthropod diversity, we used a generalized linear mixed model (GLMM), with "publication" as random variable, and change in plant diversity, duration of treatments, whether the study was experimental or descriptive (see below) as fixed variables. Also, we included an account of the difference in grazing intensity between compared treatments for studies making more than one comparison: "1" indicates a small difference in density (for instance low vs. moderate density), whereas 3 indicates a large difference in density (e.g. ungrazed vs. intensively grazed), "2" was used for comparisons in between. Analyses were performed in R 2.14.1 (R Core Team, 2013), with use of the nlme library (Pinheiro et al. 2012).

### 3.3.2 Dataset description

Our search yielded 145 studies assessing the effects of large herbivores on arthropod communities published between 1940 and 2013, sometimes in combination with other management types. An overview of the taxonomic and geographic focus of all 145 studies is given in Fig. 3.1. Ground beetles, butterflies and grasshoppers have been studied most extensively, while other, sometimes extremely species-rich groups, such as parasitic Hymenoptera, (non-syrphid) flies and aphids have received virtually no attention (Fig. 3.1a). More than half of the studies assessed only one taxonomic group, with less than 25% of studies assessing more than two arthropod taxa (Fig. 3.1b). The number of years that arthropods were sampled during these studies varied: about half of the studies sampled a single year while only two studies sampled more than 8 years (Fig. 3.1c).

The majority of grazing studies were conducted in Europe (>65%; Fig. 3.1d), where domestic grazer populations are often managed for nature conservation purposes. In North America (21%) and Africa (5%) grazing studies are also regularly conducted, often focusing on the effects of wild herbivores, sometimes in comparison to domestic livestock. Studies from Oceania, Asia and South America are rare, although several studies from these continents have been published on grazing effects in wood- or shrublands. The vast majority of studies was published after the year 2000 (Fig. 3.1e).

Studies of the effects of large herbivores on arthropod diversity can roughly be divided into two types: controlled experimental approaches and historic studies. In controlled experiments, a comparison was made between experimental plots receiving (randomly assigned) treatments differing in stocking density or grazing species (e.g. Gibson et al. 1992; Dennis et al. 1997; Joern 2005; Rickert et al. 2012). These include studies using exclosures to exclude some or all vertebrate herbivores within sites (Morris 1967; Fisher Barham & Stewart 2005; Gómez & González-Megías 2007). Controlled

experiments usually ran less than ten years (although some impressive examples of long-term experimental grazing research exist) and generally had a relatively small number of replicates. In historical studies, effects of grazing were generally compared among a number of sites with different herbivore species or different herbivore densities (e.g. Smith 1940; Kruess & Tscharntke 2002a,b; Nickel & Hildebrandt 2003). Here, the number of replicate sites and the geographical extent were usually larger, but the sites did not necessarily have a constant grazing pressure or identical starting conditions. In our database, experimental and historical studies were represented approximately equally.

Out of the total of 145 studies, 24 could be used for the quantitative analysis comparing grazing effects on arthropod and plant diversity, giving a total number of 53 data points. Twenty of these studies were conducted in Europe, one in Africa, and two in the Americas. Ecosystems ranged from prairies and savannas to coastal salt marshes and alpine grasslands. Both experimental and descriptive approaches were represented and responses of arthropod and plant richness in two to five grazing treatments were compared.

### 3.3.3 Results

Across all studies, arthropod diversity responded significantly negatively to an increase in grazing intensity ($\mu$ =-0.17±0.03, t=-5.45, p<0.001, Fig. 3.2a), with over 80% of the data points showing a decrease in richness. Plant diversity, however, did not show a significant response to grazing ($\mu$ =0.01±0.03, t=-0.04, p=0.70), with approximately as many positive responses as negative ones (Fig. 3.2b). When the two effects were compared, the response of arthropod diversity was significantly more negative than that of plant diversity ($\mu$=-0.16±0.03, t=4.45, p<0.001, Fig. 3.2a). GLMM revealed a significant, but weak positive relation between the responses of plant and arthropod diversity ($\beta$ = 0.40±0.13, t=3.13, p=0.004, $R^2$=0.13, Fig. 3.2b), but no significant effect of study duration, experimental type or differences in herbivore densities. This number of non-significant variables might be explained by a low sample size or the difficulty of comparing ecosystems with variable grazing histories and productivity.

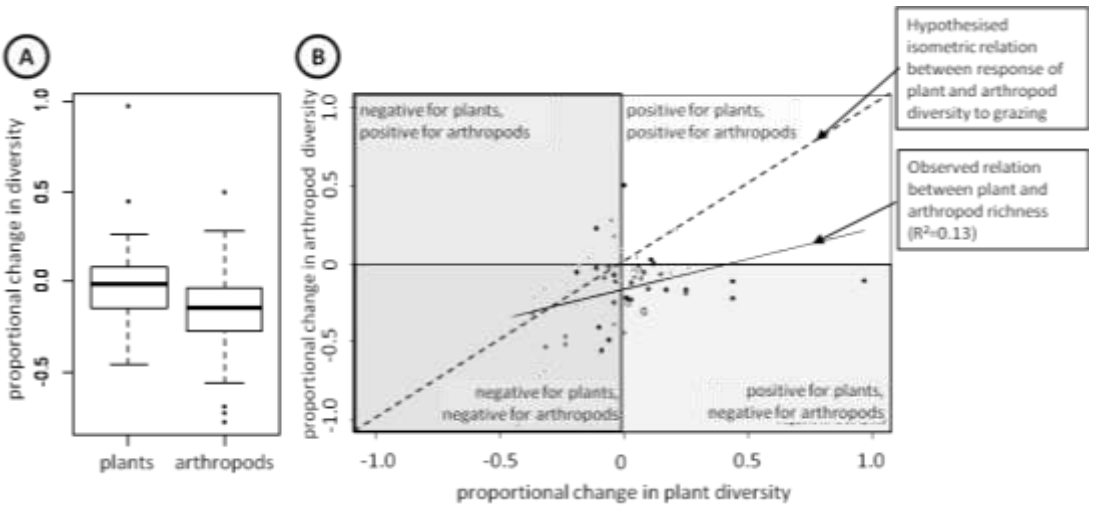


**Figure 3.2.** Comparison of the response of plant and arthropod diversity to an increase in grazing intensity (a), and the relationship between these responses (b). Data were extracted from 24 published studies between 1940 and 2013 reporting on the effects of grazing on both plant and arthropod diversity, supplemented with data obtained from several authors. * denotes a significant difference ($p < 0.05$) according to paired student's t-test.

## 3.4 Mechanisms underlying grazing effects on arthropod diversity

The quantitative analysis in the previous section showed that 1) the prevailing effect of large herbivore grazing on arthropod diversity is negative, 2) within studies arthropod diversity responds more negatively to grazing than plant diversity, 3) response of plant diversity to grazing is a poor predictor for response of arthropod diversity and 4) there are large differences between studies in the effects of grazing on arthropod diversity. In order to understand these differences we will focus on the potential mechanisms by which large herbivores affect arthropod species. Fig. 3.3 shows a conceptual framework of direct and indirect pathways through which herbivores can affect arthropods. The impact of these pathways on arthropod diversity is mediated by the three ecological determinants of the populations that constitute a community: (1) abiotic conditions of the environment (including non-trophic use of biotic structures), (2) trophic resource availability and (3) predation (Chase & Leibold 2003). We use these determinants to classify the mechanisms by which arthropods are affected.

### 3.4.1 Direct effects

Large herbivores can affect arthropod diversity directly through unintentional ingestion or trampling (Fig. 3.3, path1), but also by supplying resources for specialized groups such as dung feeders and scavengers (Fig. 3.3, path 2). Each of these mechanisms will be discussed here briefly.

Large herbivores frequently ingest arthropods as a byproduct of their forage intake. Such unintentional predation can lead to reduced arthropod population sizes (Gómez & González-Megías 2007; Bonal & Munoz 2007; Van Noordwijk et al. 2012), but the potential consequences at the community level have rarely been measured. Gómez & González-Megías (2007) demonstrated large differences between guilds of herbivorous insects in susceptibility to unintentional predation. While endophagous insects (living within plant structures) often were ingested by large herbivores, ectophagous insects (living on plants) were generally not affected. Some of these species have been shown to avoid ingestion by dropping off the plant when detecting the presence of large vertebrates (Gish et al. 2010; Ben-Ari & Inbar 2013). These differences in the vulnerability to incidental ingestion between arthropod guilds suggest large potential shifts in arthropod communities.

Large herbivores also cause direct disturbance while moving through their habitats, which is most evident in the form of trampling living vegetation, litter and soil (Cumming & Cumming 2003; Hobbs 2006, Fig. 3.3, path 1). Knowledge on the extent to which this affects arthropods is limited, but there is some observational (Chappell et al. 1971; Bayfield 1979; Bonte & Maes 2008; Woodcock & Pywell, 2009) and experimental (Duffey 1975) evidence that trampling by herbivores or humans mostly negatively affects population sizes and diversity of arthropods. It is not always clear, however, whether these effects resulted directly from direct trampling on arthropods, or indirectly, through changes in soil, litter or plant characteristics (see also section 3.2 and 3.3). Duffey (1975) demonstrated convincingly that even low frequencies of 5-10 treads per month on litterbags affected the fauna highly detrimentally, and Chappell et al. (1971) showed large decreases in faunal abundance between lightly and heavily trampled calcareous grasslands. For less mobile arthropods, such as caterpillars, but also for large dung beetles (Negro et al. 2011) trampling could be an underestimated direct source of mortality (Fig. 3.2, path 1). Additionally, frequent disturbance by large herbivores may decrease habitat suitability for arthropods. This may again be of extra importance for less mobile species that could experience difficulties in returning to their host plants, like many larval insects (Dennis et al. 1998; Kruess & Tscharntke 2002a).

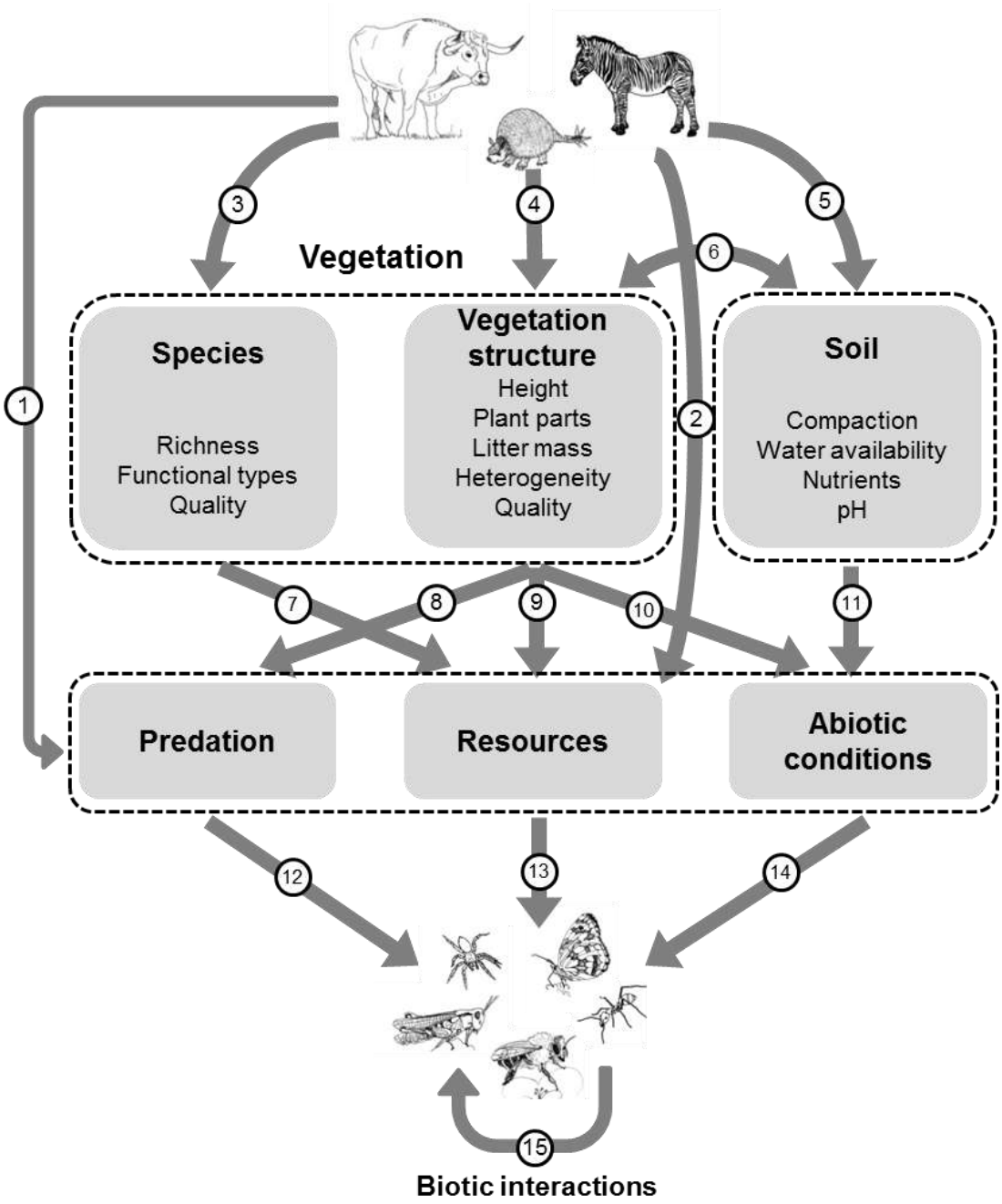


**Figure 3.3.** A conceptual framework of mechanistic pathways by which large herbivores directly and indirectly affect arthropod diversity. Arrows represent mechanisms, with numbers referring to the accompanying box (next page) and in the main text. The first row of boxes represents biotic and abiotic conditions that are modified by large herbivores, the second row of boxes represents the three determinants of arthropod populations togenther forming the communities.

**Paths of figure 3**

**Path 1:** direct effects: trampling and unintentional predation (section 3.4.1).
**Path 2:** direct effects: dung, carcasses, blood, live tissue (section 3.4.1).
**Path 3:** Increase or decrease in plant diversity and changes in functional groups, the direction of which depends on their density and the properties of the ecosystem (section 3.4.3).
**Path 4:** changes in vegetation structure: lowering of vegetation height through defoliation and changes in horizontal heterogeneity resulting from selectivity (section 3.4.2).
**Path 5:** Changes in soil conditions (PH, bulk density) (section 3.4).
**Path 6:** Changes in soil conditions can affect vegetation characteristics (section 3.4.4)
**Path 7:** A change in plant diversity can affect diversity of associated insect herbivores. (section 3.4.3).
**Path 8:** A reduction in vegetation height can increase predation risk by vertebrate predators (section 3.4.2).
**Path 9:** Direct competition for resources between the base of the arthropod food-web and large grazers (section 3.4.2).
**Path 10:** A reduction in vegetation hight increases surface temperatures, but decreases shelter from climatic extremes and essential structures for egg deposition or web construction (section 3.4.2).
**Path 11:** changing soil properties may affect insects that spend part of their lives undergound (section 3.4.4).
**Path 12, 13, 14:** the combined changes in abiotic conditions, resources and predation determine the effects on each arthropod species, and will therefore affect arthropod populations and communities.
**Path 15:** due to the interactions between arthropod species, changes in species' abundances might have cascading effects on other species, with ultimate effects on total species richness (section 3.4.5).

Conversely, large herbivores may have positive effects by directly supplying resources to arthropods in the form of dung, carcasses, blood and living tissue (Fig. 3.2, path 2). Studies investigating the effect of dung on arthropod communities mostly focused on dung beetles, despite the fact that also termites (Freymann et al. 2008) and various fly families feed on dung. Not surprisingly, these studies often report positive effects of large-herbivore presence on dung beetle diversity and abundance (Lumaret et al. 1992; Verdu et al. 2007; Jay-Robert et al. 2008), but too high herbivore densities may be detrimental to dung beetle abundance and diversity (Jankielsohn et al. 2001; Negro et al. 2011). Differences in dung beetle diversity between livestock grazing and natural herbivore assemblages have been reported to be small, although community composition can differ between areas with different herbivore assemblages (Jankielsohn et al. 2001; Numa et al. 2012). Effects of livestock management on dung feeding fauna is also strongly influenced by the use of antiparasitic medication, which has highly detrimental effects on dung feeding fauna (Wall & Strong 1987; Madsen et al. 1990) and decomposition rates (Wall & Strong 1987; Beynon et al. 2012).
Although it is obvious that the presence of herbivores may enhance the diversity of scavenging and parasitic arthropods, field studies showing such patterns are scarce (Barton et al. 2013). Evidence has been presented that a deer carcass can be a hotspot for biodiversity compared to the surrounding forest (Melis et al. 2004) and that the presence of large herbivores can decrease populations of mice and their fleas (McCauley et al. 2008). For these arthropod groups, human influence may be of extra importance, because in many grazed ecosystems, the resources that these species depend on are highly managed. For instance, removal of carcasses and treatment with anti-parasitic medication are very common in European semi-natural grasslands. Also targeted extermination of livestock parasites has eradicated several species from parts of their former range (e.g. Wilson 1986; Vreysen et al. 2000) but introductions of livestock outside their native range have probably enhanced the spread of their parasites even more (e.g. Scholl 1993). The anthropogenic changes in large herbivore densities, with in its most dramatic form extinctions of species, will almost certainly lead to co-extinctions of their parasites (Dunn et al. 2009) and scavengers.

In conclusion, the direct effects of large herbivores on arthropod diversity are potentially manifold and sometimes obvious, but are, with the exception of dung beetles, poorly quantified. Nevertheless, the overall impact on arthropod diversity of these direct effects is probably small in comparison to the indirect effects, as we will see in the next sections.

### 3.4.2 Vegetation-structure mediated effects

The most prominent effect caused by large herbivores is defoliation, leading to a decrease in vegetation height and structural complexity (Fig. 3.2, path 4). Most plants can tolerate defoliation to some extent by resorting to dwarf growth, vegetative spread, or by fast regrowth. Repeated defoliation and trampling can lead to changes in plant species composition (path 3), which will be discussed in section 3.3. For arthropods, short and tall vegetation types provide different abiotic conditions, food resources and predation risk (Fig. 3.3, path 8,9,10). The currently emerging insights how these differences affect arthropod diversity will be outlined below.

The abiotic conditions arthropods are exposed to differ vastly between short and tall vegetation (Fig. 3.3, path 10). When a vegetation is permanently grazed short and bare soil is exposed, this often leads to a warmer microclimate in the vegetation and higher soil temperatures, which are essential for the larval development of various thermophilous arthropods such as many grasshopper and butterfly species (e.g. Thomas et al. 1986; Cherrill & Brown 1992; Bourn & Thomas 2002; Roy & Thomas 2003). Moreover, several species require bare, exposed soil for egg deposition (e.g. tiger beetles) or nesting (e.g. solitary bees). Tall and dense vegetation, on the other hand, can act as a temperature buffer, with relatively cool temperatures during the day and benign temperatures at night or in winter (Luff 1966; Dennis et al. 1994), or provide shelter from extreme climatic conditions such as droughts or (periodical) floods (Pétillon et al. 2008). It also offers complex three-dimensional structures for web-building spiders, for species that deposit eggs in or on plants (e.g. some grasshopper species) and offers hiding and stalking opportunities for predatory arthropods in the canopy (e.g. crab spiders, praying mantes).

Resource availability also differs between tall and short vegetation (Fig. 3.3 path 9). Tall vegetation possesses aerial structures, like flowers and stems, and the removal of these structures is logically detrimental to their consumers, such as pollinators (Gomez 2003) and insects developing in flower heads and fruits (Morris 1967; Völkl et al. 1993; Gómez & González-Megías 2007). Tall, ungrazed, vegetation is usually also accompanied by a dense litter layer, providing food for detritivores and their predators. Large herbivores consume large quantities of plant biomass that will therefore not enter the detrital food-web. Litter additions have indeed been shown to increase abundance of predatory arthropods (Langellotto & Denno 2004).

Conversely, short-grazed vegetation offers resources in the form of short-statured plants, that many specialized herbivorous insects depend upon (Van Klink et al. 2013; Thomas et al. 1986), but also in the form of nutrient-rich regrowth. After defoliation, the young leaves often have higher nutrient contents than older plant parts (McNaughton 1976; Ydenberg & Prins 1981). All else being equal, herbivorous insects react positively to an increase in resource quality (White 1993; Ritchie 2000), which sometimes leads to species attaining plague densities (Onsager 2000). Positive effects on arthropod diversity, however, have thus far not been shown. Other plant species, especially in dry, unproductive systems, respond to defoliation by producing secondary compounds that are unattractive to large herbivores, but usually also for herbivorous arthropods (Vicari & Bazely 1993; Nykanen & Koricheva 2004). Specialist arthropods, however, have often co-evolved with their host plants in such a way that they tolerate or even profit from the secondary compounds that are produced after defoliation by large herbivores (Poelman et al. 2009).

Furthermore, predation risk is modulated by vegetation height (Fig. 3.3, path 8). Large-eyed predators, such as some ground beetle species (Morris 2000), but also vertebrate predators, such as birds (Belovsky et al. 1990), hunt more efficiently in short vegetation or on bare ground. Tall vegetation may thus protect arthropods from predation, although the densities of arthropod predators, such as spiders, are known to increase with vegetation complexity (Langellotto & Denno 2004).

Taken together, tall, complex vegetation should generally provide more food resources (Lawton 1983), lower predation risk (Belovsky et al. 1990) and more opportunities for coexistence of arthropods than short vegetation, for instance through vertical niche differentiation (Denno 1980). Indeed, a positive relation between vegetation biomass and arthropod diversity is often reported (Duffey 1962; Luff 1966; Woodcock et al. 2007; but see Joern 2005; Woodcock & Pywell 2009). Consequently, arthropod diversity has often been found to decrease with increasing densities of large herbivores (e.g. Dennis et al. 1997; Kruess & Tscharntke, 2002a, 2002b; Pöyry et al. 2004; Rickert et al. 2012). Some arthropod species, however, depend on short vegetation with patches of bare soil (e.g. Joern & Lawlor 1981). It is therefore likely that heterogeneous vegetation, consisting of a patchwork of short and tall vegetation should harbour highest arthropod diversity.

Large herbivores can, under specific circumstances, enhance vegetation heterogeneity. They are usually not distributed homogeneously over the landscape, and exhibit spatial selectivity in their behaviour, such as feeding, defecation and wallowing (dust-bathing, which creates sparsely vegetated patches (Collins & Barber 1985)). Spatial heterogeneity in feeding behaviour can lead to a patchy vegetation structure of short and tall vegetation if (1) herbivores forage selectively, (2) herbivore density is too low to consume all vegetation and (3) there is a positive feedback between large herbivores and the quality of their food (Adler et al. 2001). Resulting heterogeneity in vegetation structure can then lead to heterogeneity of other ecosystem processes (McNaughton 1984; Hobbs 1996). This is most likely to occur in productive ecosystems (Hobbs & Swift 1988). Conversely, if these conditions are not met, or when high underlying abiotic heterogeneity is already present, grazing is more likely to decrease heterogeneity (Adler et al. 2001).

Although arthropod diversity would be expected to be highest in heterogeneous grasslands, evidence for this relationship is remarkably scarce. Joern (2005) showed a positive relation between grasshopper diversity and grazing-induced heterogeneity in vegetation height, but results from other studies show other relationships (Van Klink et al. 2013; Dennis et al. 1998). Moreover, some studies report highest vegetation heterogeneity to occur after cessation of grazing, and consequently find highest arthropod diversity under these conditions (e.g. Kruess & Tscharntke 2002a; Pöyry et al. 2006).

To complicate matters, the effects of grazing on vegetation structure vary across spatial scales (WallisDeVries et al. 1999; Adler et al. 2001). Grazing may, for example, lead to a more homogenous vegetation structure at a small scale, while simultaneously leading to heterogeneity at a larger scale (Adler et al. 2001). Such divergent effects of herbivores on vegetation heterogeneity may obscure general effects on arthropods.

Heterogeneity in vegetation structure caused by large herbivores may not only be expressed spatially, temporal heterogeneity is also likely to occur. This may be caused by seasonal variation in plant growth, but also by temporal variation in grazing pressure due to seasonal herbivore migrations or active management (Fryxell & Sinclair 1988; Bischof et al. 2012). The range of spatial and temporal scales at which grazers can affect heterogeneity severely complicates field measurements of the effects on arthropod diversity. An increased understanding of the spatial and temporal scales at which grazing affects vegetation heterogeneity and knowledge of how scale affects the availability of resources and abiotic conditions for arthropods will greatly enhance our understanding of the impact of large herbivores on arthropod diversity.

### 3.4.3 Vegetation-community mediated effects

Large herbivores often have profound effects on plant diversity (Fig. 3.3, path 3) and plant ecologists have a long history of studying these (Olff & Ritchie 1998). In general, effects of herbivores on plant diversity tend to be positive in wet, productive systems and negative in dry, infertile ones (Olff & Ritchie 1998; Proulx & Mazumder 1998; Bakker et al. 2006; Lezama et al. in press.). Moreover, some of the most plant-species rich ecosystems in the world are traditionally grazed grasslands in Europe (Wilson et al. 2012). A decrease of grazing, therefore often leads to a decrease in plant diversity, as light competition causes exclusion of short-statured plant species (Grime 1973).

Arthropod (consumer) diversity has been hypothesized to be correlated with plant (producer) diversity (Murdoch et al. 1972; Tilman 1986), and experimental increases of plant diversity have indeed been shown to increase arthropod diversity (Siemann et al. 1998; Haddad et al. 2009), abundance (Haddad et al. 2001), functional group richness (Siemann et al. 1998; Rzanny & Voigt, 2012) and food-web complexity (Scherber, Eisenhauer, Weisser, *et al.*, 2010; Rzanny & Voigt 2012). Moreover, this relation was not only found for diversity of herbivorous insects, but also for predators (Haddad et al. 2009) and parasitoids (Ebeling et al. 2012). However, in experimental grazing research this interrelation between plant and arthropod diversity has rarely been supported. In fact, several researchers showed a negative response of arthropod diversity to grazing even when plant diversity increased (Kruess & Tscharntke 2002a; Pöyry et al. 2004), and the generality of these results is corroborated by our quantitative review. The response of plant diversity to grazing therefore seems to be a poor predictor for the response of arthropod diversity.

Obviously, the loss of host plants due to grazing or a lack thereof will lead to the co-extinction of its specialist herbivores. However, the presence of a plant species does not guarantee suitable conditions for its specialist herbivores. This may be due to the presence or absence of certain required plant parts (Morris 1967) or the size of the plant (Lawton 1983), but also to microclimate (Thomas et al. 1986), or isolation from the closest source population (Kruess & Tscharntke 1994). Moreover, tall-statured and widespread plant species generally harbor a richer fauna of specialist insect herbivores than short-statured plant species (Lawton & Schroder 1977; Strong et al. 1984). This implies that with a lack of grazing, replacement of a short-statured host plant will cause a relatively small loss in diversity, while the gain of tall-statured species can potentially cause a large increase.

Another obvious way by which large herbivores modify the composition of plant communities, is by changing the relative abundance of different plant functional groups (Fig. 3.3, path 3). For instance, in wet, productive systems, grazing can increase the cover of palatable, grazing tolerant plant species (often grasses) (McNaughton 1984), whereas in arid systems it can increase the abundance of unpalatable shrubs (Archer et al. 1995). In temperate systems, both intensive grazing and cessation of grazing can cause an increase in the relative cover of grasses at the expense of forbs. Consistent with these observations, polyphagous (grass-feeding) insects have been found to increase under intensive grazing (Nickel & Hildebrandt 2003) as well as after cessation (Littlewood 2008). Similarly, the diversity of both insect-pollinated plants and flower-visiting insects can be affected positively (Vulliamy et al. 2006), negatively (Potts et al. 2009) or not at all (Batáry et al. 2010) by large herbivores. This suggests that shifting abundances of different functional plant groups as a result of grazing can have a large impact on herbivorous and flower-visiting insects and that these shifts may better explain changes in arthropod communities in response to grazing than plant diversity per se.

### 3.4.4 Soil-mediated effects

Large herbivores can have a strong impact on soil properties, with some of the most consistent outcomes being altered levels of soil nutrients, pH values, water availability (Milchunas & Lauenroth 1993; Bakker et al. 2009) and increased soil compaction (Trimble & Mendel 1995) (Fig. 3.1, path 5). Changes in soil conditions can lead to changes in plant communities (Liddle 1997) (Fig. 3.3 path 6), but can potentially also have direct effects on aboveground arthropods (Fig. 3.3 path 11).

Although the effects of grazing on belowground fauna are strong (Bardgett & Wardle 2003; Beylich et al. 2010), few studies report soil-mediated effects of herbivores on aboveground arthropods. Many species best known for their aboveground appearance, such as clickbeetles and crane flies, spend part of their life cycle below ground, as egg or larva. During these developmental stages, arthropods have been shown to react to changes in soil nutrients (Larsen et al. 1996; Goulet 2003; Oliver et al. 2005), pH (Van Straalen & Verhoef 1997; Goulet 2003) and moisture level (Goulet 2003), which can all be altered by large herbivores. Indications that herbivore-mediated changes in soil properties may affect aboveground fauna have so far only been reported for rove beetle communities (Hofmann & Mason, 2006) and some ant species (Bestelmeyer & Wiens 2001). The generality of these effects is, however, as of yet poorly known.

### 3.4.5 Effects on interactions between arthropod species

Like all organisms, co-occurring arthropod species interact in myriad ways, including through resource competition, predation and mutualistic interactions (Fig. 3.3, path 15). Food-webs are complex in nature, and often, changes in one trophic level can have unforeseen consequences for another trophic level or guild (Schmitz 2011). Experimental evidence for the way in which large herbivores can alter relations between arthropod species is scarce (but see Vanbergen et al. 2006).

In grasslands there is a great potential for bottom-up driven diversity control, as suggested by the strong relationship between vegetation complexity and arthropod diversity (section 3.2). An increase in abundance or diversity of herbivorous insects and detritivores can potentially increase the diversity of higher trophic levels, as was shown in plant diversity manipulation experiments (e.g. Scherber et al. 2010). From grazing experiments, so far only correlative evidence is available, showing similar changes in the diversity of herbivorous and predatory taxa to changes in grazing pressure (Gibson et al. 1992; Kruess & Tscharntke 2002b; Báldi et al. 2012). Moreover, the diversity of parasitic Hymenoptera was found to be well correlated to overall diversity (Anderson et al. 2011), suggesting that these potentially indirectly respond to herbivore-mediated changes in diversity of lower trophic levels. Still, causal relations explaining these changes have not yet been mapped in a context of grazing.

There is also potential for changes in top-down processes controlling diversity, since large herbivores can affect the abundance and diversity of predatory arthropods, which then might affect the diversity of lower trophic levels. Evidence for the importance of this process in grasslands is, however, extremely limited, and increased predator abundance may in fact enhance the diversity of lower trophic levels (Sanders & Platner 2007). To better understand these complex relations, there is a strong need for food-web approaches in grazing research, with a good potential for path analysis (e.g. Scherber et al. 2010).

Finally, it is possible that grazing alters competitive outcomes between arthropod species from the same trophic level. For plants, it is well established that grazing strongly alters competitive relationships (Hobbs & Huenneke 1992; Olff & Ritchie 1998), but for arthropods, evidence is scarce. The importance of competitive exclusion in arthropod communities has been debated for decades (Lawton & Hassell 1981; Denno 1995). Although there is now ample evidence that resource competition and competitive exclusion do occur between herbivorous insects (White 1993; Denno 1995; Reitz & Trumble 2002), it remains unclear how important these processes are in structuring natural communities in a field setting. Since the vast majority of arthropod species exploit different resource bases, the importance of competition between species in limiting diversity is probably small (Strong et al. 1984). Therefore, the disruption of competitive hierarchies by large herbivores is unlikely to affect arthropod diversity (Fuentes & Jaksic 1988). Disentangling the relative importance of all these processes remains a formidable future challenge.

## 3.5 Synthesis

### 3.5.1 Why is arthropod diversity so often negatively affected by grazing?

Ultimately, the mechanisms through which large herbivores affect arthropods are mediated by three key main components of arthropod population regulation: predation, trophic resource availability and abiotic conditions (Fig. 3.3). In the presence of large herbivores, (unintentional) predation and direct mortality of arthropods are likely to increase, which is especially likely to affect sedentary arthropods (section 3.1). These direct effects will be negative for diversity if mortality rates are high, but neutral if arthropod populations can be maintained.

The total trophic resource availability for arthropods will be reduced as herbivores consume plants and litter, which form the base of the arthropod food-web (section 3.2). Therefore, overall arthropod abundance is likely to be decreased under grazing. Given the large body of theoretical (Fisher et al. 1943) and empirical evidence (Kruess & Tscharntke 2002a; Pöyry et al. 2006) showing a positive relationship between abundance and diversity of organisms, defoliation by large herbivores can be expected to be negative for arthropod diversity. However, plant diversity is often increased by grazing, creating opportunities for a wider group of specialist herbivores (section 3.3). Also for

species such as dung beetles and parasites resource abundance will increase with grazing (section 3.1).
Large herbivores often strongly change the abiotic environment experienced by arthropods (section 3.2). Such changes will be positive for some species and negative for others. Overall effects on diversity therefore depend on the habitat requirements of the species present in the local species pool and the interactions of large herbivores with prevailing (climatic) conditions.
Taking all these effects together, the variation in biotic (e.g. dung and plant species) and abiotic (e.g. microclimate and habitat complexity) conditions *may* be increased by large herbivores (sections 3). Therefore, arthropod diversity *can* be increased by large herbivores if the following conditions are met: 1) grazing causes an increase in biotic and abiotic heterogeneity, 2) this increase in heterogeneity occurs at such a spatial and temporal scale that it can be exploited by the species present in the local species pool and 3) this positive effect of increased heterogeneity is large enough to compensate for the negative effects of direct mortality and resource competition between arthropods and large herbivores. This combination of conditions is most likely to occur at low densities of herbivores, because direct mortality and resource competition are minimal, while variation in (a)biotic conditions is most likely to increase (see section 3.2).
High densities of large herbivores are likely to always be detrimental to overall arthropod diversity, although some specific arthropod groups may profit. This is indeed supported by most empirical studies (e.g. Kruess & Tscharntke, 2002a,b; Rickert et al. 2012). Studies reporting otherwise (Vulliamy et al. 2006; Yoshihara et al. 2008) have all studied flower-visiting insects, which may not spend their whole life-cycle in the study environment and may not represent overall arthropod diversity (Vessby et al. 2002; Oertli et al. 2005).

### 3.5.2 Why is arthropod diversity more negatively affected by grazing than plant diversity?

The difference between plants and arthropods in response to grazing can be understood by considering the mechanisms by which both groups are affected. Three differences between plants and arthropods emerge to explain the contrasting response to grazing.
First, plant diversity is generally increased by grazing through a decrease in light competition, and an increase in colonization by new species (Olff & Ritchie 1998). Since there is no evidence for an important role of competition in limiting arthropod diversity (section 3.5), it is unlikely that large herbivores can cause any type of competitive release on arthropod communities. Conversely, the majority of species at the base of the arthropod food-web (herbivores and detritivores) compete directly for resources with large herbivores, as outlined in the previous section. This competition is highly asymmetrical, and can lead to competitive exclusion and decreased population sizes (Gomez & Gonzalez-Megias 2002), which is likely to reduce arthropod diversity.
Secondly, the habitat requirements of plants and arthropods operate at different spatial and temporal scales (Bourn & Thomas 2002). Plants are sedentary and need a specific set of conditions that are all met at one spot. Arthropods generally have distinct phases in their life-cycle, which often need different site conditions (e.g. warm microclimate and abundant host plants for larval development and nectar for adult life-stages). Especially immature stages have been found to have a narrow niche and limited dispersal ability to actively find suitable habitat patches (Bourn & Thomas 2002). For arthropods to survive, the requirements of all life-cycle stages must be met within the area the species can travel. This means that single arthropod species often need a certain level of habitat heterogeneity (creating favorable microclimatic conditions and food resources for all life stages) at a specific spatial scale to survive. Plant species, on the contrary, can thrive in fairly homogeneous grasslands as long as their specific habitat requirements are met. As more intensive grazing management generally decreases habitat heterogeneity (see section 3.2) this inevitably negatively affects many arthropod species, even if the requirements of single life-stages are still met. In addition, the life-cycle of many arthropod species is strictly synchronized (Zaslavski 1988). This means that the habitat conditions for each life-cycle stage must be present at exactly the right time of year, making arthropods especially

sensitive to the timing of grazing (Lenoir & Lennartsson 2010; Carvell 2002; van Noordwijk et al. 2012)
Third, plants are more plastic in their response to grazing than arthropods are. Plants can often survive (periodical) high trampling and defoliation through dwarf growth, vegetative spread and belowground storage of resources. Arthropods generally do not have such back-up strategies. Some arthropods can attempt to escape unfavorable conditions by dispersal (Berggren 2004), but they can only disperse over limited distances where they have to find favorable conditions again. This difference in vulnerability to grazing between plants and arthropods has strong implications for nature conservation.

### 3.5.3 Implications for arthropod conservation management

Most grassland types worldwide depend on the presence of large herbivores to prevent succession to scrub or forest (Hobbs 1992). In most of these grasslands herbivore densities are (strongly) influenced by human intervention including active management, exploitation, agricultural activities and abandonment of former agricultural practices. This will have profound impacts on these grasslands and their biodiversity, including arthropod diversity. Grazing goals, and hence decisions on stocking densities and other human interventions, vary widely over grazed ecosystems. A major part of grazed systems is being used for livestock grazing, where production of meat or other animal products is the primary goal. A much smaller area is grazed for conservation purposes. Here management priorities vary from a focus on maintenance of diverse herbivore assemblages in African savannahs (Mbano et al. 1995), to restoration of natural processes on the North American prairies (Sanderson et al. 2008) and a focus on maintenance of high (plant) diversity in European semi-natural grasslands (WallisDeVries 1998; Ostermann 1998). In agricultural grazing systems effects on (arthropod) diversity are generally not considered in decision making. Indeed, studies investigating the effects of livestock grazing in agricultural systems usually report negative impacts on diversity (Smith 1940; Forbes et al. 2005; Xie et al. 2008) and abundances (King & Hutchinson 1983) of arthropods. Also in natural and semi-natural grasslands, arthropods are not always given high priority, but awareness of the importance of arthropods is growing among conservationists, as is attention for arthropods in conservation and restoration research (Fig. 3.1e). Our review highlights that specific attention for arthropods is essential for their conservation, as arthropods are generally more sensitive to grazing than plants. Therefore we highly recommend that arthropod species richness is monitored in addition to botanical composition when evaluating grazing management,
Although grazing is essential to conserve species-rich grasslands on the long run, we have shown that increased grazing intensity quickly becomes detrimental to overall arthropod diversity. On the other hand, high plant species richness is often best attained under moderate grazing regimes (Olff & Ritchie 1998; Wilson et al. 2012) and many thermopilous insects, including many butterflies depend on favourable microclimates (Bourn & Thomas 2002) created by more intensive grazing (see section 3.2). Both plants and thermophilous butterflies typical of semi-natural grasslands have become especially threatened due to increased eutrophication and abandonment of historic farming practices (Ostermann 1998; van Swaay et al. 2010) and hence have attained special conservation interest (van Swaay et al. 2010; EU Habitats Directive). This creates potential for conflict between the requirements of plant diversity, threatened arthropod species and maintenance of high overall arthropod diversity (see for example Negro et al. 2013). To avoid such conflicts and to meet the requirements of as many species as possible, the conservation or creation of a heterogeneous habitat is imperative. Low densities of herbivores provide the best chance of attaining this objective (see section 4.1), but so far no evidence has been presented that a single management regime can accommodate all species in a local species pool (Dennis et al. 1997,2001). Therefore, it has been suggested that arthropod diversity can best be conserved at the landscape scale by maintaining grasslands under different types of management in close proximity (Dennis et al. 1997; Morris, 2000; Kruess & Tscharntke 2002; Rickert et al. 2012). In addition to such spatial variation, temporal heterogeneity can be created by using rotational grazing with periods (weeks to decades) of grazing alternated with periods of cessation. This creates periods in which the negative effects of grazing (direct mortality and

resource competition) are absent (Morris 1967), while still creating high plant species diversity and an open vegetation structure. Rotational grazing has been shown to be successful for arthropod conservation in several habitats (Morris et al. 2005; Farruggia et al. 2012), but needs additional research in many others. Offering variation in grazing intensity and timing and considering effects of grazing on a landscape scale may also be a feasible approach to increase arthropod diversity in agricultural landscapes, especially where agricultural fields are interspersed with semi-natural habitats (Tscharntke et al. 2012).

Whether specific species survive under a given grazing regime inevitably depends on the match between their habitat requirements and the timing, scale and intensity of grazing. While low intensity grazing and variation of grazing intensities at the landscape scale will benefit overall arthropod diversity, more detailed grazing regimes will be required in cases where a specific suite of target species has been set. In these cases, a fruitful approach to finding the optimal grazing regime is to analyse the life-cycles of these species (Verberk et al. 2013). This approach has been advocated for conservation purposes (Van Noordwijk et al. 2012), but can also be used to actively suppress populations of pest species (Onsager 2000).

## 3.6 Next steps

From this review, clear patterns explaining the patterns of arthropod diversity in grazed ecosystems have emerged. Analysing the mechanisms affecting arthropod diversity responses to grazing has revealed causes for the variation in these as previously reported, and for the overall negative response of arthropods to (intensive) grazing. Our study has also identified a number of issues that remain poorly understood and require further research. Although we have argued that a positive effect of large herbivores on arthropod diversity can mostly be expected at low herbivore densities, empirical evidence remains scarce, and more experimental testing is needed. In particular we need to expand our knowledge of the specific conditions under which large herbivores have a positive effect on arthropod diversity, for example by directly comparing a number of promising low intensity grazing regimes. As we have demonstrated that spatial and temporal heterogeneity in (a)biotic conditions are crucial to arthropod diversity, these aspects need special attention. It has become apparent that there are large differences between arthropod taxa in their response to grazing. Therefore, multi-taxon studies are highly desirable, preferably conducted over multiple years to account for weather effects and population dynamics. In addition, a great deal can be learnt from smaller experimental studies targeting single mechanisms (e.g. incidental ingestion, effects of soil compaction or effects of plant diversity). To add to our current knowledge, these experiments should especially focus on effects of these mechanisms at the community level (to what extent are diversity and composition affected). Helpful approaches in this respect include 1) trait-based approaches, demonstrating which traits determine to what extent arthropod species are affected by the studied mechanisms and 2) integrated food-web studies, demonstrating the importance of bottom-up, top-down and competitive interactions in shaping arthropod communities in grazed ecosystems. A food-web approach could also be used to link above- and belowground effects of large herbivores. Finally, to understand differences in responses of arthropod diversity to grazing between ecosystems it is important to be able to compare in situ grazing pressure between studies and ecosystems. This is currently hampered, for example due to differences in ecosystem productivity and land-use history. An account of the percentage net primary productivity consumed by large herbivores should improve comparability, and aid future syntheses.

## 3.7 Conclusions

1) The vast majority of published studies on the effects of grazing on arthropods were conducted in Europe and North America, and focus on a small number of arthropod taxa. Studies demonstrating effects on overall arthropod diversity are virtually lacking.

2) Responses of arthropod diversity to grazing are highly variable, but arthropod diversity is often more negatively affected than plant diversity. Moreover, plant diversity is a poor predictor for arthropod diversity in grazed ecosystems. Therefore, we strongly recommend to consider the specific

requirements of arthropods and to include arthropods in monitoring schemes evaluating the effects of grazing.
3) Three main processes drive the impact of large herbivores on arthropod diversity: Unintentional predation and disturbance have a negative effect on population sizes and therefore diversity of most arthropod groups. Positive direct effects, like availability of resources such as dung and carrion, will only benefit a small number of arthropod species.
4) Defoliation by large herbivores will cause a reduction of resource abundance for the base of the arthropod food-web (herbivores and detritivores) and also reduces habitable space for species dependent on tall vegetation structures. This will generally have a negative effect on diversity.
5) Large herbivores can under specific conditions increase both plant diversity and structural heterogeneity of the vegetation. This increase in resource heterogeneity can increase arthropod diversity, but only if its positive effects are large enough to compensate for the negative effects large grazers have outlined above.
6) Conservation strategies aiming at maximizing heterogeneity, such as low intensity grazing, maintenance of different types of management in close proximity, or rotational grazing regimes, are most likely to conserve or restore arthropod diversity.

## 3.8 Acknowledgements

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# Chapter 4: Trait similarity patterns within grass and grasshopper communities: multitrophic community assembly at work

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## 4.1 Abstract

Trait-based community assembly theory suggests that trait variation among co-occurring species is shaped by two main processes: abiotic filtering, important in stressful environments and promoting similarity, and competition, more important in productive environments and promoting dissimilarity. Previous studies have indeed found trait similarity to decline along productivity gradients. However, these studies have always been done on single trophic levels. Here, we investigated how interactions between trophic levels affect trait similarity patterns along environmental gradients. We propose three hypotheses for the main drivers of trait similarity patterns of plants and herbivores along environmental gradients: i) environmental control of both ii) bottom-up control of herbivore trait variation and iii) top-down control of grass trait variation.

To test this, we collected data on the community composition and trait variation of grasses (41 species) and grasshoppers (53 species) in 50 plots in a South African savanna. Structural Equation Models were used to investigate how the range and spacing of within-community functional trait values of both grasses and their insect herbivores (grasshoppers; Acrididae) respond to (i) rainfall and fire frequency gradients and (ii) the trait similarity patterns of the other trophic level.

The analyses revealed that traits of co-occurring grasses became more similar towards lower rainfall and higher fire frequency (environmental control), while showing little evidence for top-down control. Grasshopper trait range patterns on the other hand were mostly directly driven by vegetation structure and grass trait range patterns (bottom-up control), while environmental factors had mostly indirect effects via plant traits. Our study shows the potential to expand trait-based community assembly theory to include trophic interactions.

## 4.2 Introduction

Inspired by Jared Diamond's original idea of trait based 'assembly rules', many ecologists have adopted a trait-based approach in order to search for common principles in the assembly of local communities. According to this approach, the processes governing community assembly can be seen as 'filters' that either allow or exclude species with certain functional traits from a species pool to enter a local community (Diamond 1975; Drake 1991; Weiher & Keddy 1995a; McGill et al. 2006). Two processes are classically considered to be central in trait-based community assembly: abiotic filtering, that is, the exclusion of species that do not tolerate a particular abiotic stress (such as high temperatures), and competition filtering, that is, the exclusion of inferior competitors (Weiher & Keddy 1995a; Weiher et al. 1998). Both these processes operate through functional traits that do or do not allow a species to survive and successfully compete in a community, consequently resulting in non-random patterns of within-community trait similarity (Weiher et al. 2011). The effects of these processes on trait variation are generally expected to be opposite, with abiotic filters causing more restricted trait ranges among co-occurring species than expected by chance (hereafter called trait underexpansion, Fig. 4.1a) (Weiher & Keddy 1995a; Weiher et al. 1998; Cornwell & Ackerly 2009), while competitive exclusion of species with too similar niches results in communities of species with a higher spacing of trait values that expected by chance, hereafter called trait overdispersion

(Macarthur & Levins 1967; Pacala & Tilman 1994; Cornwell & Ackerly 2009, but see Mayfield & Levine 2010; Scheffer & van Ness 2006) (Fig. 4.1a).

Over the last decades, the effects of these filters on trait similarity patterns have been studied in various ecosystems and species groups, with mixed results, varying from random trait variation in communities, trait overexpansion and trait underdispersion (reviewed in Weiher et al. 2011). These contrasting outcomes can arise because of differences among habitats in the extent to which different community assembly processes dominate (Pausas & Verdu 2008; Cornwell & Ackerly 2009; Anderson et al. 2011) or because different processes act upon the assembly of different traits (Prinzing et al. 2008; Ingram & Shurin 2009). However, despite the quite trivial notion that all organisms can consume and/or can be consumed by other organisms, and that these trophic interactions generally depend on traits (e.g. Crawley 1989; Diaz et al. 2001), no study has ever simultaneously investigated within-community trait similarity patterns of two or more coexisting, interacting trophic levels. We thus still do not know how relevant such trophic interactions are in explaining trait similarity patterns within communities. Therefore, in this study we investigate whether trait-similarity patterns of communities of different trophic levels respond similarly to environmental gradients and whether trait variation patterns of one trophic level can result in similar patterns in another trophic level.

When studying such questions with a multitrophic approach, we suggest that it is better to replace the concept of abiotic filtering with fundamental niche filtering as the process that restricts trait ranges in communities. This is because in herbivores and predators, the availability of resources (food types, which are often not abiotic) can impose important restrictions on the fundamental niche of a species, restricting the set of species that can competitively interact in communities. In addition, the realized niche of species is generally not only restricted by competition filters, but can also be limited by predation filters. So from a multitrophic perspective, we suggest that dispersal, fundamental niche, competition and predation filters interact within and across trophic levels (Fig. 4.1b).

In this multitrophic perspective, different fundamental niche filters can operate on the community assembly of different taxonomic or trophic groups, even in the same environment (Fig. 4.1b). For example, low soil pH may filter out some plant species that cannot tolerate soil acidity, while not directly acting upon herbivore community assembly. On the other hand, certain disturbances, such as traffic noise, can have a large impact on animals, but not on plants.

In addition, the assembly processes on different trophic levels may interact (Fig. 4.1b), where the resulting trait patterns of communities of one trophic level affect the community assembly of other trophic levels. These interactions can have different directions, where alternative hypotheses have analogies to classic food web theories on top-down versus bottom-up regulation of populations. Filters on plant community assembly can be the structuring force, where the resulting plant trait variation imposes various filters (arrows H1 – H4 in Fig. 4.1b) on possible herbivore traits. This we term the Bottom-up Control Hypothesis of Community Assembly. For example, environments with infertile soils can filter out plant species that demand high nutrient levels for their leaves, The resulting plant community then filters out small herbivore species that require high quality leaves (bottom-up restriction of trait expansion patterns). On the other hand, in areas where light competition results in plant species with a high spacing in leaf size, allometric scaling laws predict similar spacing patterns in the body size of herbivores (Ritchie & Olff 1999), providing a hypothetical example of bottom-up control of trait dispersion patterns. Alternatively, filters on herbivore community assembly can determine which plant species with which traits can occur in the community (arrows Pl1-Pl4 in Fig. 4.1b), which we call the Top-down Control Hypothesis of Community Assembly. For example, the proximity of rivers or lakes can determine whether herbivores, like hippo, requiring the vicinity of water, can persist in a certain area. The resulting herbivore communities will filter out tall plant species (Diaz et al. 1991) (top-down effects on trait expansion patterns). The top-down control of trait *dispersion* patterns is also possible, as multiple (e.g., different-sized) herbivores can suppress superior light competitors and thereby promote the coexistence of plant species with different traits (Olff & Ritchie 1998).Similar arguments can be developed for how interactions between herbivores and predators affect trait-based community assembly, and for indirect interactions between predator and

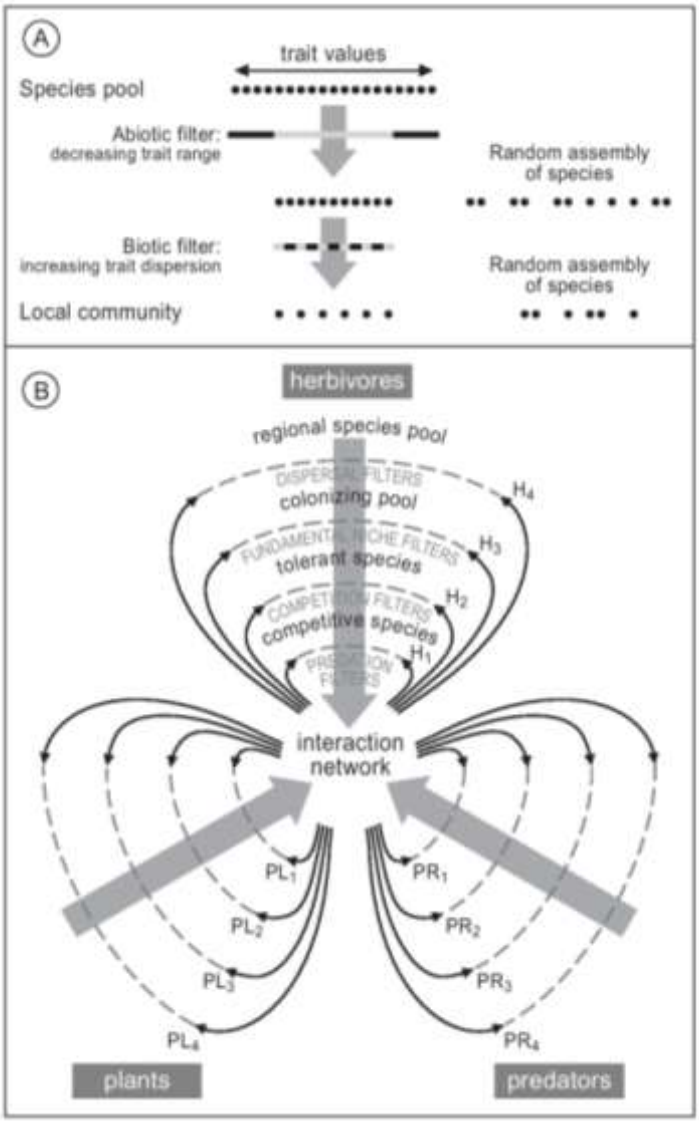


**Figure 4.1. A.** The classic conceptual idea for the assembly of communities *sensu* Diaz et al. (1998) and Weiher et al. (1998). On the left the community is formed through an abiotic filter and resource partitioning, while on the right a random selection of species from the species pool assembles in the community. The abiotic filter allows only species with trait values within a certain range that is smaller than the trait range of randomly assembled species, resulting in trait underexpansion. Competition and the consequent resource partitioning between species results in a relatively large evenness of trait distances within this range (hereafter trait overdispersion), compared with the trait distance of randomly assembled communities.
**B.** A newly proposed multitrophic extension of community assembly theory. This figure depicts community assembly at three trophic levels: producers, herbivores and predators. Here, the process of community assembly goes through a dispersal, fundamental niche, competition and predation filter. These filters determine which species can coexist in the local community or 'interaction network'. They visualize if the occurrence of a species is restricted by its ability to sufficiently disperse, find its basic resources and tolerate the prevailing abiotic conditions, compete for resources and withstand predators and diseases. The interaction network which results from these filters is not a static result, but shows a feedback on them. For example, by providing shelter, the presence of certain plant species may affect fundamental niche filtering of herbivores. Also, predation or herbivory may influence competitive filtering between plants or herbivores respectively.

plant traits (Fig. 4.1b). In line with classical theory, it is also possible that fundamental niche filters on different trophic levels are mostly abiotic in nature (e.g. frost) and overrule the importance of trophic interactions in determining community assembly (all feed-back arrows in Fig. 4.1b are unimportant), which we term the Environmental Control Hypothesis of Community Assembly.
Here, we investigate the within-community trait expansion and dispersion patterns of two trophic levels, grasses (Poaceae, focusing on aboveground traits) and co-occurring herbivorous grasshoppers (Acrididae), over a rainfall and fire frequency gradient in a South-African savanna landscape. The aim of this study is to investigate (1) whether and how within-community trait similarity patterns differ between grasses and grasshoppers, (2) how their trait similarity patterns affect each other (top-down vs. bottom-up), and (3) how trait similarity varies along environmental gradients (environmental control). In this, we are able to compare the results of the classic approach of studying how abiotic and biotic filters affect community assembly with our novel multitrophic framework.

# 4.3 Methods

## 4.3.1 Study Area

Field work was carried out in Hluhluwe-iMfolozi Park (HiP), an 89,665 ha nature reserve in Kwazulu-Natal, South Africa (S4, Fig. S4A). This park is characterized by a high local and regional habitat heterogeneity, consisting of a mosaic of closed forests, open *Acacia* woodlands, bunch grasslands and grazing lawns (Whateley & Porter 1983; Owen-Smith 2004). This heterogeneity results from gradients in altitude (ranging from 50 to 500 m), rainfall (ranging from 650 mm $year^{-1}$ till 985 mm $year^{-1}$ in the higher altitude areas), fire frequency (mean fire return intervals from 2 to 6 years), hydrology and soil heterogeneity (S4, Fig. S4A and S4B). Grass height is mainly determined by the interplay between fire, grazing by ungulates and soil fertility (Cromsigt & Olff 2008).
Throughout the park, 50 grassland plots of 10 x 10 meters, with a woody coverage below 15% were chosen that together covered the whole rainfall and fire frequency gradients. The plots were at least 25 m away from each other and were therefore considered as independent replicates (see further documentation of this assumption in S4). In these plots, we performed a one-time sampling of all grass and the herbivorous species and we measured vegetation height. Fieldwork was carried out from April through June 2008.

## 4.3.2 Environmental data

Rainfall data from January 2001 through December 2007, from 17 rain gauge stations, more or less evenly distributed across the study area, were used to construct an annual amount of rainfall map with the Kriging interpolation method, which takes spatial covariance of rainfall patterns into consideration. The Kriging interpolation was performed using the four nearest rain gauge stations, in Arc-GIS v 9.3.1 (see for the map Fig. S4A). GIS data on a 200 m x 200 m scale about the fire frequency were available through digitized fire maps that were annually drawn between 1956 and 2004 by the park management authorities (Fig. S4B).

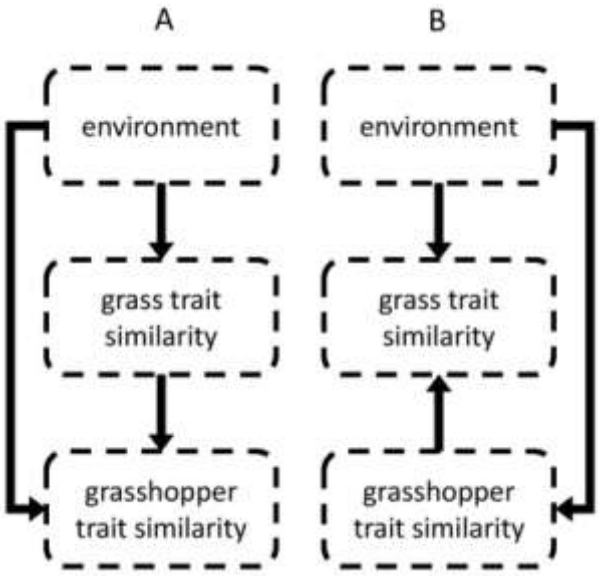


**Figure 4.2.** Hypothesized relationships between environmental gradients and within-community grass and grasshopper trait similarity patterns. Arrows indicate causal relationships, boxes indicate major categories of variables. In the Bottom-up Control Model (**A**), within-community grass trait similarity (either expansion or dispersion) patterns are affected by the abiotic environment, while within-community grasshopper trait similarity patterns are affected by both the abiotic environment and by grass trait similarity patterns. In the Top-Down Control Model (**B**), within-community grass trait similarity patterns are affected by both the abiotic environment and by within-community grasshopper trait similarity patterns. Within-community grasshopper trait similarity patterns are only affected by the abiotic environment.

### 4.3.3 Grass and grasshopper data

Within each plot, the canopy height (i.e. height of highest leaf) of the grass layer was measured at one location in which the height was more or less equal to other places in the plot, and therefore representative. Vegetation height measurements were rounded to tens of centimeters, unless the height was lower than 5 cm, in which case height was rounded to 2.5 cm. Furthermore, five flowering individuals from each grass species were picked for the measurement of grass height, leaf surface area (LA) and specific leaf area (SLA) in the HiP Research Station. These traits were chosen because they represent different allocation strategies to drought stress (LA, Givnish (1987)), relative growth rate (SLA), and competitive ability for light (height, Weiher et al. 1999), and because they have been shown to respond to community assembly processes (e.g. Anderson et al. 2011).

At the HiP research station, the canopy height of individual grasses was measured following Cornelissen et al. (2003). Leaf material was taken to the Groningen University (Netherlands). There, leaf blades were re-wet between two pieces of tissue paper. After re-wetting, leaves were put on a scanner together with a black reference square (3 x 3 cm) for calibration. With the software package ImageTool v3 (UTHSC, Antonio, TX, USA) the surface area (LA) of the leaves was measured. Afterwards, the leaves were dried in an oven at 70C for at least 24 h. After one day of cooling, the leaf weight was measured with a scale to 0.001 g precision and SLA ($cm^2$ leaf / g leaf) was calculated. Within the same plots adult grasshoppers of the family Acrididae were collected with sweep nets for 15 minutes and taken to the HiP research station for species identification and morphological measurements.

In the HiP Research Station, grasshoppers were identified to species level and digital photographs of the grasshoppers together with a ruler for calibration were taken. The ImageTool v3 software

(UTHSC, Antonio, TX, USA) was used to measure total body length and wing length. Relative wing length of each grasshopper species was calculated by taking the unstandardized residuals from a regression analysis with species wing length as the response variable and species total body length as the predictor variable. Positive residuals thus indicated a relatively large wing length, while negative residuals indicated a relatively small wing length. Total body length and relative wing length were chosen, because these traits reflect variation in diet requirements and resource partitioning (total body length, Belovsky (1997)) and energy allocation to dispersal or stress avoidance (wing length, Harrison 1980).

### 4.3.4 Within-community trait similarity

Grand mean trait values of species were used for calculating within-community trait similarity patterns. This assumes that there are significant intrinsic species differences, which was indeed the case: with, depending on the trait, between 47 (grass LA) and 83 % (grasshopper body length) of the total variation explained by species identity. For both grasses and grasshoppers the scaled observed trait range (OTR: highest minus lowest observed species trait value divided by the mean trait value) was calculated for each trait within each plot. Furthermore, we calculated the observed trait evenness (OTE) for each trait within each plot. OTE was defined as $\frac{1}{sdT+1}$ in which sdT is the standard deviation of distances between adjacent trait values. It was therefore a measure for the evenness of differences between adjacent co-occurring trait values, with values close to zero indicating a very low evenness and a value of one indicating maximum evenness. To test whether the OTR and OTE values in plots were higher or lower than expected by chance, i.e. under- or overexpanded (trait range) or under- or overdispersed (trait evenness), we used two different null models. To study trait expansion, we performed 10.000 random draws from the species pool (see Fig. 4.2A, top right) without replacement, at each observed species richness. The species pool was defined as all the species sampled in this study. The chance of drawing a certain species from the species pool was proportional to the number of plots in which it occurred, to prevent false positives caused by an over-representation of rare species with extreme trait values in the randomized communities. Of the 10.000 random draws performed at each species richness, we calculated the mean scaled randomized trait range (RTR). Unscaled residual trait range (ReTR) or trait expansion for each plot was defined as: ReTR = OTR – RTR, in which RTR is the randomized trait range for random draws with the same richness as in the observed plot. Positive ReTR values thus indicated trait overexpansion (i.e. a higher within-community trait range than expected by chance), while negative values indicated trait underexpansion.

In the second null model, for each plot we took 10.000 random community draws under constrained conditions: from the species pool (again defined as all the species observed during this study) S – 2 (in which S is the observed species richness of the plot) species with trait values that fell within the unscaled observed trait range were selected. Again, the chance of a species being selected for the random community was proportional to its relative frequency in the species pool. Furthermore, the species with the highest and lowest trait value in the given observed community were also present in the randomized community. This way, we constructed new random communities in which the species richness and trait range remained the same as in the observed communities, while the distances of trait values within that range could change (bottom right part of Fig. 4.1a). Then, we calculated the average trait evenness of the 10.000 random draws for each plot (randomized trait evenness or RTE). For each plot, unscaled residual trait evenness (ReTE) or trait dispersion was calculated with the formula ReTE = OTE – RTE. Positive ReTE values thus indicated trait overdispersion (i.e. a higher within-community trait evenness than expected by chance), while negative values indicated trait underdispersion.

Finally, A Wilcoxon Signed-Rank test was used to test for overall significance of trait expansion and dispersion across plots. All calculations and the construction of null models were performed with R-2.9.2 (R Development Core Team 2009). For a more detailed description of the development of the null models and the assumptions underlying them, see S4.2.

### 4.3.5 Identifying drivers of trait similarity patterns

For both trait expansion and trait dispersion (residual trait range and residual trait evenness), two *a priori* chosen competing conceptual path models (or construct models, that represent theoretical hypotheses without all the mechanistic details) were developed: the Bottom-up Control Model and the Top-down Control Model (Fig. 4.2). In the Bottom-up Control Model, the abiotic environment affects the grass trait similarity patterns, while grasshopper trait similarity patterns are affected by both the abiotic environment and grass trait similarity patterns (Fig. 4.2a). Thus, the Bottom-up Control Model tested for both bottom-up processes and environmental control processes explaining variation in trait similarity patterns. In the Top-down Control Model, grass trait similarity patterns are affected by both the abiotic environment and by grasshopper trait similarity, while grasshopper trait similarity patterns are only affected by the abiotic environment (Fig. 4.2b). Thus, the Top-down Control Model tested for both top-down processes and environmental control processes explaining variation in trait similarity patterns.

Structural Equation Models (SEMs) (Grace 2006; Grace et al. 2010) were run to test the fit of the construct models with the Amos 17.0 software package (Arbuckle 2007) using a maximum likelihood approach. These models were used because they allow for the testing of both direct and indirect effects of landscape variables on trait similarity patterns (Grace et al. 2010). Furthermore, because of their multivariate nature, in which most variables affect and are being affected by several other variables, with SEMs one can compare the strength and significance of opposite pathways (see e.g. Grace et al. 2007), which allowed in our case to compare the relative strength of bottom-up and top-down processes in community assembly. In total, we ran four models: a Bottom-up Control Model describing how trait expansion patterns of grasshopper communities are driven by trait expansion patterns in plant communities (Bottom-up Control Model for trait expansion), the competing Top-down Model for trait expansion, in which grass trait expansion patterns were affected by grasshopper trait expansion patterns, and an equivalent Bottom-up and Top-down Control Model for explaining community trait dispersion patterns. We chose to do the analyses for trait expansion and dispersion patterns separately, because these trait similarity variables represent separate (relatively independent) ecological mechanisms. Furthermore we chose to keep our saturated SEMs relatively simple to minimize the risk of overfitting (see for S4.3 for a more elaborate discussion on this choice). However, to test for the robustness of the models with expansion and dispersion analysed separately, we also ran a Bottom-up Control Model and a Top-Down Control Model with all trait similarity variables included (shown in S4.3).

**Table 4.1**. Overall patterns in grass and grasshopper trait range and evenness relative to null models. 'no. plots > 0' indicates the number of plots in which the observed trait range or evenness was lower than the average of 10.000 random draws with the same species richness. Bold *P* values indicate a significant pattern (Wilcoxon signed-rank test). Abbreviations: LA – Leaf Area, SLA – Specific Leaf Area, ReTR – Residual Trait Range, ReTE – Residual Trait Evenness.

| | ReTR | | | ReTE | | |
|---|---|---|---|---|---|---|
| | no plots > 0 | *P* | interpretation | no plots > 0 | *P* | interpretation |
| **grass** | | | | | | |
| LA | 19 out of 50 | **0.006** | underexpansion | 32 out of 50 | **0.018** | overdispersion |
| SLA | 13 out of 50 | **0.001** | underexpansion | 19 out of 50 | **0.049** | underdispersion |
| height | 20 out of 50 | **0.005** | underexpansion | 22 out of 50 | 0.743 | |
| **grasshoppers** | | | | | | |
| length | 20 out of 50 | 0.244 | | 21 out of 50 | 0.463 | |
| relative wing size | 14 out of 50 | 0.251 | | 25 out of 50 | 0.824 | |

**Table 4.2.** *T*-test results showing for each path explaining a trait expansion pattern in Fig. 4.3 whether this pattern is either caused by response in maximum trait values or minimum trait values in communities to a change in the predictor variable.

| | minimum value | | | maximum value | | |
|---|---|---|---|---|---|---|
| path | *b* | *t* | *P* | *b* | *t* | *P* |
| rainfall → LA ReTR | 0.004 | 3.51 | < **0.001** | 0.021 | 5.45 | < **0.001** |
| fire freq. → height ReTR | 1.344 | 3.86 | < **0.001** | 1.351 | 4.60 | < **0.001** |
| fire freq. → length ReTR | -0.031 | -0.73 | 0.466 | 0.833 | 4.15 | < **0.001** |
| veg. height → length ReTR | 0.004 | 0.47 | 0.641 | 0.170 | 4.36 | < **0.001** |
| height ReTR → wing ReTR | 2.756 | 1.54 | 0.130 | -6.722 | -2.33 | **0.024** |
| LA ReTR → wing ReTR | -3.365 | -2.00 | 0.051 | 8.282 | 3.13 | **0.003** |

As environmental variables, we included the average amount of rainfall per year and fire frequency. As the grass variables we used the ReTR or ReTE of grass LA, SLA and canopy height. Furthermore, we included vegetation height in the models. As the grasshopper variables, we used ReTR or ReTE of grasshopper length and relative wing size. We started our analyses with saturated models, in which all grass- and grasshopper variables were predicted by the environmental variables and in which all grasshopper variables were predicted by all grass variables (Bottom-up Control Models), or vice versa (Top-down Control Models). Furthermore, in the saturated models, fire frequency was predicted by annual amount of rainfall. For a graphical overview of the models, see Fig. S4G and S4H in S4.3. Starting with the saturated models, we developed final models using the following stepwise procedure: (1) correlations significantly improving the fit of the model based on a modification index above 4.0 were added; (2) paths with P-values above 0.05 (chi-square test) were omitted; and (3) variables that were not causally connected with any other variable were omitted. Our final models thus only contained significant paths between variables. Overall model fit was assessed using the $\chi^2$ statistic and the associated *P*-value. Overall model fit was considered adequate if the associated *P*-value was above 0.05, indicating that the null hypothesis (i.e. the SEM) could not be rejected. AIC values were then used to compare the fit of competing final models (i.e. Bottom-up and Top-down Control Models). For the paths of the final SEM explaining variation in trait expansion patterns, we then tested with simple *t*-tests whether the paths linked with trait expansion patterns were either caused due to a relationship between the predictor value and (a) the maximum value or (b) the minimum value of the given trait. Thereby we investigated whether the predictor value either constrained the upper or lower limit of the given trait in local communities.
Furthermore, we also ran Multiple Regression Models with grass- and grasshopper trait expansion and dispersion variables as the response variables and rainfall and fire frequency as the predictor variables, to compare our SEM results with results from more traditional approaches (for more details, see S4.4).

# 4.4 Results

In total, in our plots we found 41 grass species and 53 grasshopper species across our 50 plots. Species richness ranged from 3 to 13 species per plot for grasses, with a median value of 7, and from 3 to 16 species per plot for grasshoppers, with a median value of 7.

## 4.4.1 Overall trait expansion and dispersion patterns

Across our 50 sampling locations, observed LA, SLA and grass canopy height values had significantly lower ranges than communities from the null model. Therefore these traits were underexpanded, or, in local communities less overall variable (difference between lowest and highest trait value) than expected from random community assembly (Table 4.1). For grass height values we found significant overdispersion across our plots (Table 4;1), indicating limiting similarity. Within-community SLA values were significantly underdispersed (Table 1). Observed within-community LA dispersion patterns did not differ from the null model (Table 4.1). Furthermore, neither of the

grasshopper traits showed within-community expansion or dispersion patterns differing from random community assembly (Table 4.1).

### 4.4.2 Structural Equation Models

For explaining variation trait expansion patterns across sites, the final Bottom-up Control Model (Fig. 4.3, S4.3 for statistics of all paths) is the model that fitted the data best (AIC = 39.957, $\chi^2$ = 5.957, $P$ = 0.867 vs. AIC = 42.159, $\chi^2$ = 10.169 and $P$ = 0.601 for the final Top-Down Control Model). This model shows that variation in grass traits could mainly attributed to environmental differences across sites. Vegetation height increased with annual amount of rainfall. Also LA trait expansion increased with rainfall, due to a relatively higher increase of maximum LA values than minimum LA values in communities (Table 4.2). Height trait expansion decreased with fire frequency (Fig. 4.3), due to an stronger increase of the mean grass height relative to the range in grass height with higher fire frequencies (Table 4.2).

For variation across sites in grasshopper trait expansion, we found that it was mostly bottom-up regulated by grass variables and mostly indirectly by environmental variables. Grasshopper body length trait expansion increased with vegetation height and fire frequency, due to an increase of maximum body length values with vegetation height and fire frequency (Table 4.2). Relative wing size trait expansion increased with LA trait expansion and decreased with grass height trait expansion, since maximum relative wing size values were highest in areas with a high LA trait expansion and a low grass height expansion (Table 4.2).

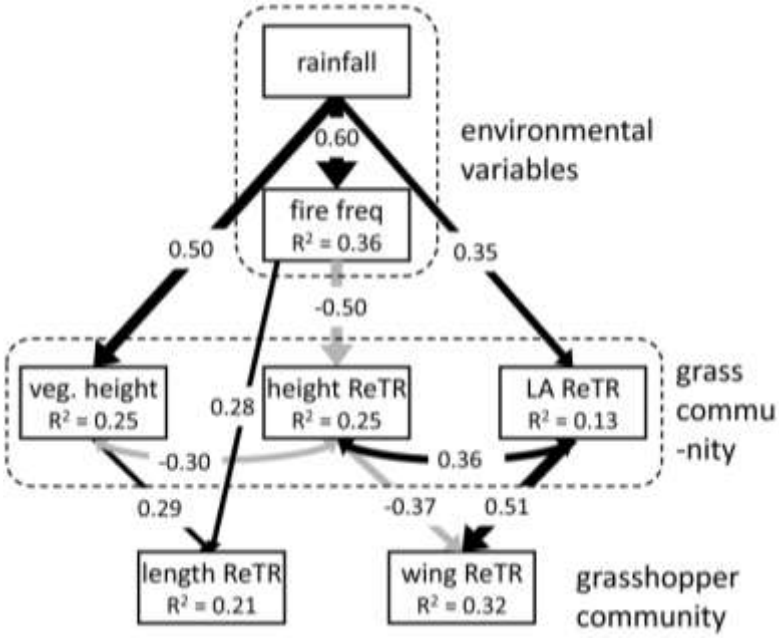


**Figure 4.3.** Final model ($\chi^2$ = 5.957, d.f. = 11, $P$ = 0.876, AIC = 39.957) explaining the trait range expansion of grasses and grasshoppers, after the non-significant paths were omitted. Single-arrow connectors represent direct effects of one variable on another, while double-arrow connectors represent correlations. The thickness of the arrows indicates the interaction strength. Black paths indicate positive effects, while gray paths indicate negative effects. The *R*-square indicates the total proportion of variation of the given variable that could be explained by the model.

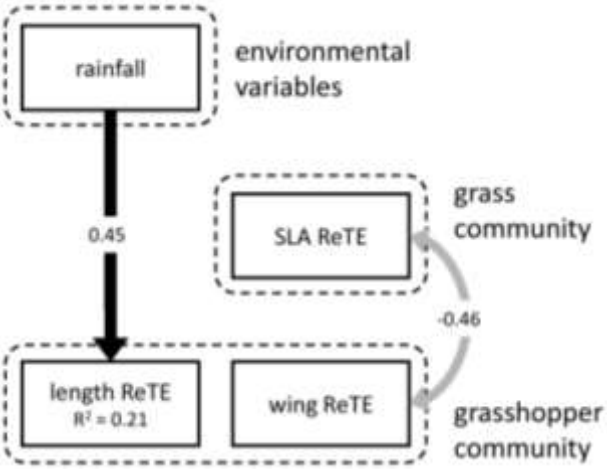


**Figure 4.4.** Final model ($\chi^2$ = 2.582, d.f. = 4, $P$ = 0.630, AIC = 14.582) explaining the trait dispersion of grasses and grasshoppers, after the non-significant paths were omitted. Single-arrow connectors represent direct effects of one variable on another, while double-arrow connectors represent correlations. The thickness of the arrows indicates the interaction strength. Black paths indicate positive effects, while gray paths indicate negative effects. The *R*-square indicates the total proportion of variation of the given variable that could be explained by the model.

Variation in trait dispersion across sites was similarly explained by the final Bottom-Up and Top-Down Control Model (Fig. 4.4, S4.3 for statistics of all paths) (AIC = 14.582, $\chi^2$ = 2.582, $P$ = 0.630). The SEMs shows that grasshopper body length dispersion was positively affected by annual rainfall, while grasshopper relative wing size dispersion is negatively correlated with SLA trait dispersion (Fig. 4.4).

In addition, we developed two saturated SEMs (Bottom-up and Top-down Control) that simultaneously included trait expansion *and* dispersion variables (S4.3, Fig. C5 and C6). Of these two models, the final Bottom-up Control SEM of trait similarity patterns performs best and shows qualitatively the same results as the previous SEMs (S4.3, Fig. C7). This suggests that the SEMs, in which dispersion and expansion patterns were analyzed separately, produced robust conclusions.

The SEM results yielded different outcomes than traditional approaches, in which grasshopper body length expansion was best explained by fire frequency ($t$ = 2.570, $P$ = 0.013), grasshopper wing expansion by rainfall ($t$ = 2.371, $P$ = 0.022), grasshopper body length dispersion by rainfall ($t$ = 3.530, $P$ < 0.001) and grasshopper wing could not be explained by any of the environmental variables (S4.4).

## 4.5 Discussion

Our data provide the opportunity to evaluate if the proposed multitrophic framework of trait-based community assembly leads to different conclusions than analyses based on the classic idea of abiotic and competitive filters within trophic levels. We therefore tested if trophic interactions are additionally important in trait-based community assembly. According to classic trait-based community assembly theory (without trophic interactions), trait underexpansion patterns are expected in communities that are mainly structured by abiotic filtering, while trait overdispersion is expected when competition is important (Cornwell & Ackerly 2009). When using the standard analyses as used in other studies, we found significant underexpansion patterns for all grass traits but not for grasshopper traits, while significant overdispersion patterns were hardly found. This would suggest that abiotic filtering was the most important process in structuring grass communities, with only a

limited role for interspecific competition in community assembly, and no explanation for observed within-community grasshopper trait similarity patterns. Furthermore, traditional Multiple Regression Analyses that tested for effects of variation in environmental variables on grasshopper trait similarity patterns suggested that variation between sites in within-community grasshopper trait similarity patterns were directly driven by variation in rainfall and fire frequency. However, we also used multivariate SEM analyses as a statistical method to test for the importance of trophic interactions in community assembly. This showed that variation in grass trait expansion patterns were explained by rainfall and fire, while grasshopper trait expansion patterns were largely explained by vegetation structure and grass expansion patterns (supporting the Bottom-Up Control Hypothesis of Community Assembly) and therefore only indirectly by environmental gradients. This shows the importance of trophic interactions on trait-based community assembly, and that our SEM analyses provide conclusions that cannot be picked up with the classic methods of analysis used in the literature so far.

Given the nature of our study system, it was to be expected that bottom-up controls (plant trait distributions determine grasshopper trait distributions) were more important than top-down effects. Previous work has shown the importance of fire, large mammalian herbivores and rainfall in regulating plant community composition (e.g. Cromsigt & Olff 1998) and furthermore, invertebrate herbivores generally have a lower impact on plant communities than vertebrate herbivores (Crawley 1989) and are often bottom-up regulated (e.g. Haddad et al. 2001). However, our main objective was to show *that* trophic interactions next to environmental factors can affect the trait distribution on different trophic levels. The overall importance of top-down versus bottom-up controls on trait distributions at different trophic levels would require extensive meta-analyses of data from different ecosystems.

As our SEMs showed, trait expansion patterns in grasses were determined by environmental factors. These environmental factors can drive the observed trait expansion patterns in the grasses in different ways. In accordance with other studies, plant trait ranges became less constrained in more 'benign' environments: LA expansion became larger in high rainfall areas in accordance with Cornwell & Ackerly (2009). In dry areas, only species with small leaves occurred, while both species with large and small leaves occurred in high rainfall areas. Large leaves evaporate relatively more water (Givnish 1987) and therefore our results are possibly the outcome of the inability of species with large leaves to survive in dry, low rainfall areas. Fire had a negative effect on the canopy height trait expansion of the grasses. This is in line with Pausas & Verdu (2008), who showed that fire reduced trait space in mediterranean tree communities. The limited range in grass canopy height values in areas that burn relatively frequently can possibly be explained by the fact that only tall grass species occur in such areas, which have a higher root/shoot ratio (Anderson et al., unpublished manuscript), allowing them to resprout after fires. The more constrained range in grass trait values in the more stressful dry and frequently burnt areas suggests that in those areas, fundamental niche filtering through abiotic conditions is important, only allowing those species that can persist drought and fire.

Trait variation in grasshoppers, on the other hand, seemed to be more structured by trophic interactions and only indirectly by environmental gradients. Grasshopper body length expansion was highest in areas with a tall vegetation. While less attractive for large mammalian grazers, these areas seem to support the highest quantities of food for grass-feeding grasshoppers. Relatively large insect species also require relatively large quantities of food (Belovsky 1997) and could thus be filtered out in areas with low vegetation height and low food availability. The resulting pattern of relatively high trait expansion patterns in areas with a high vegetation and low trait expansion patterns in areas with a low vegetation is an example of a bottom-up process, in which the plant community imposes a fundamental niche filter on grasshoppers (arrow H3, Fig. 4.1). But an alternative explanation is that in areas with short vegetation, relatively large and conspicuous grasshoppers are more vulnerable to avian predators preferring to hunt in open vegetation, thereby excluding large species (Belovsky et al. 1990). That would be an example of a bottom-up process in which the local plant community, by forming certain structures, imposes a predation filter on the herbivore community (arrow H1, Fig. 4.1). Grasshopper wing expansion was highest in areas with a high grass LA expansion. LA has been suggested to be low in nutrient poor sites (Givnish 1987), and areas with a high LA expansion could

thus be seen as areas with a high variation in resource quality for herbivorous insects. Some grasshopper species have strong preferences for high quality food, while other species can digest lower quality food as well (Behmer & Joern 2008). In areas with a high variation in food quality, no single food type dominates, so that more selective species should be able to disperse when their food source has been depleted, while this ability to disperse is not needed for more generalist species. Species with relatively long wings are often good dispersers (Wootton 1992), while species with short wings are not. In areas with a high variation in vegetation quality or LA one can thus indeed expect a high variation in grasshopper wing length as well, while in other areas only species with relatively short wings occur. This would be another example of a plant community imposing a fundamental niche filter on herbivores (arrow H3, Fig. 4.1), interacting with dispersal filters. Surprisingly, we found that expansion of relative wing length was lowest in areas with a high expansion of grass canopy height, where mainly relatively short-winged grasshoppers occur (Table 4.3). A possible explanation for this may be the following. Expansion in relative wing length of grasshoppers may be seen as variation in ability to disperse. Low expansion may thus be caused by a lack of species with good dispersal ability, since species with a high relative wing length were rare in areas where grass height trait expansion was high. High expansion of grass canopy height implies high structural complexity of the vegetation, which is in general beneficial for food availability of insects (Haddad et al. 2001). Species with low dispersal ability or low relative wing length may therefore be expected to dominate in such favorable habitats.

We hardly found evidence for trait overdispersion for grasshopper or above-ground grass traits. Overdispersion is expected to arise from interspecific competition and the consequent resource partitioning between species within communities (Cornwell & Ackerly 2009). This would imply that grasses in this savanna do not compete very strongly with each other. Competition between grasses and trees in savannas has been extensively investigated, and has yielded important functional differences that may promote their coexistence, such as rooting depth, fire tolerance and access to nutrients (Scholes & Archer 1997; Cramer et al. 2010). However, the importance of competition in relation to trait differentiation between different grass species in savannas has been much less investigated. Furthermore, our analyses are based on above ground grass traits and will thus be most relevant for light competition. As the grass layer in our study system is often kept low by mammalian herbivores and frequent fires, it can be understood why light competition is not a main factor in structuring these plant communities. Below-ground competition among grasses probably plays a more important role, which would require the investigation of belowground traits (rooting depth, specific root length, mycorrhizal associations etc.). However, such traits are not directly, and therefore probably also less strongly, linked to grasshopper community assembly than aboveground grass traits. Therefore, it is not surprising that the multivariate multitrophic analyses did not show strong direct links between the trait dispersion of grasses and grasshoppers.

In summary, we conclude that the effects of environmental gradients on within-community trait similarity patterns can be direct or indirect, mediated by interactions among trophic levels. With our multitrophic conceptual framework for trait-based community assembly (Fig. 4.1b) and our SEM approach, we could elucidate which candidate variables were the direct and indirect predictors of trait similarity patterns of coexisting grass and grasshopper communities, showing that trophic interactions were more important than environmental control in explaining trait similarity patterns in grasshopper communities.

## 4.6 Acknowledgements

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# Chapter 5: Local and regional drivers of tree and mammal communities in the Serengeti: the role of kopjes versus rainfall

Fons van der Plas & Han Olff

## 5.1 Abstract

African savannahs are characterized by a high biological diversity. This high diversity is usually suggested to be the result of various drivers of habitat heterogeneity acting at different spatial scales. While much research in savannahs has emphasized the importance of drivers of large scale heterogeneity, the role of smaller scale geology-driven heterogeneity has been less intensively studied. Here, we investigate to which extent large scale (rainfall gradients) and small scale (the presence of rocky outcrops or kopjes) drivers of heterogeneity explain the community composition of trees and mammals in an African savannah.

Along a rainfall gradient (annual rainfall ranging from 650 mm to 750 mm), we visited 24 sites in the Serengeti National Park in Tanzania. At each site, we characterized tree and mammal communities in a plot on a kopje and in plot in the surrounding savanna matrix. Additionally, we collected and collated data on traits of the observed species with respect to fire tolerance, herbivory defense and drought tolerance of trees and diet choice and body size of all mammals >2kg.

We found that tree communities were most species rich in high rainfall areas and even more so on kopje plots. The dominant trees in these areas were, on average, less spiny and more often broad-leaved than other savanna trees. Additionally, we found that especially small and medium-sized herbivores were less abundant in high rainfall areas and on kopjes. Three other mammal species were positively associated with kopjes: baboon, elephant and rock hyrax.

While relatively small changes in tree community composition were found along the rainfall gradient, differences between the kopje and matrix plots were much larger. These differences are likely to be related to reduced herbivory and fire incidence/occurrence on kopjes. The low abundance of small and medium-sized herbivores on kopjes may be related to the high predation risk on kopjes facilitated by stalking opportunities provided by trees and rock. Even smaller-sized browser species, such as hyrax, may be able to escape predators by hiding in cracks, and likely profit from the resources provided by woody species on kopjes. We conclude that at local scales, kopjes or rocky outcrops are important drivers of tree, herbivore and predator community composition in savannahs.

## 5.2 Introduction

African savannahs are famous for their high biodiversity (Mittermeier et al. 1998). This diversity is often suggested to result from the high heterogeneity in savannahs (Scholes 1990), both at small and large spatial scales (Cromsigt 2006). Habitat heterogeneity can be important to support many specialist species, which occur only in certain habitats (Öster et al. 2007). But also for generalist species that occur in a wide range of habitats, spatial heterogeneity can be important, because individuals from such species often acquire services from different habitats in different seasons or ontogenetic stages.

**Figure 5.1.** Photographs of two kopjes in SNP. Photo left: a kopje in the southern plains, where tree growth is hardly present in the surrounding vegetation. Photo right: kopje more north in SNP, where average amount of rainfall is higher and trees are also present in the surrounding. Photos made by Han Olff.

Heterogeneity at larger spatial scales is usually driven by environmental gradients. In savannahs, spatial variation in the amount of rainfall is one of the most important environmental gradients, driving shifts in plant and animal community composition patterns (e.g. Maddock 1979; Reed et al. 2009) as well as animal migrations (Maddock 1979; Holdo et al. 2009). Also, landscape gradients in soil fertility patterns driven by variation in parent material are import, and set together with rainfall the regional diversity patterns (Olff et al. 2002). At smaller scales, other landscape elements are more important in creating heterogeneity, such as mounds created by termites (Moe et al. 2009; van der Plas et al. 2013), vegetation mosaics created by herbivores (Archibald et al. 2005) and small-scale geological heterogeneity, such as rocky outcrops or kopjes (Fig. 5.1).

However, the effects of these drivers of habitat heterogeneity on community composition are difficult to understand for several reasons. Firstly, while researchers typically only investigate changes in *species* composition patterns between different habitats, such patterns cannot be fully understood without taking *functional* differences between species into account (McGill et al. 2006). Functional traits of species are suggested to determine the habitat requirements of a species, as well as their competitive capacity and capability to tolerate or escape predation, and thus can help in the interpretation of the observed habitat associations (McGill et al. 2006). Secondly, many studies tend to focus on a single trophic level when investigating community responses to changes in habitats. However, the responses of one trophic level cannot be fully understood without considering other trophic levels, which form resources or predation risks. Thirdly, communities usually respond to several components of habitat heterogeneity at different scales which are likely to interact. Here, we study the responses of both trees and large mammals to a rainfall gradient and geological heterogeneity (the presence or absence of kopjes) in an African savannah, following a trait-based approach.

Specifically, we investigate how species richness, abundances and functional characteristics of tree and large mammal (>2kg) communities respond to two main drivers of heterogeneity: spatial variation in rainfall and the presence of kopjes. Previous studies have shown the importance of rainfall in savannahs for both plants and animals. Water availability is one of the most important limiting factors for plant growth in savannahs (Walter 1971), and it is therefore no surprise that many plant species respond strongly to rainfall gradients (Reed et al. 2009). In general, higher rainfall areas contain higher densities of trees (Scholes et al. 2002; Sankaran et al. 2005) and also more tree species (Scholes et al. 2002; 2004; Eshete et al. 2011). Rainfall has also been shown to be important for mammals. Most large herbivores require drinking water and fresh, nutritious leaves of certain plant species to feed on, and these may not be sufficiently available in areas with low rainfall or during certain seasons within a year. As a result herbivore migrations have been associated with seasonal patterns in rainfall (Maddock 1979; Holdo et al. 2009). Additionally, large scale distributions of

resident herbivores are to a large extent driven by seasonal differences in rainfall, with larger herbivores tending to be more often present in high rainfall areas, while some smaller species are more confined to drier areas due to different food quality and quantity requirements (Olff et al. 2002; Bhola et al. 2012).

In open savanna areas of the East-African rift valley, the rocky outcrops called kopjes (similar to inselbergs or buttes in other areas) are a striking habitat feature, representing old volcanic cores (Fig. 5.1). However, such kopjes have been little investigated as a driver of savannah heterogeneity, even though the relatively few studies that did investigate species assemblages on kopjes have shown that kopjes harbor many unique plant and animal species (Trager & Mistry 2003; Poelchau & Mistry 2011). Their unique habitat features are likely due several factors. First, plant growth on kopjes is mostly confined to the cracks that occur between rocks. The resulting structures allow plants to penetrate with their roots to deeper moist soil layers. This is in contrast to the surrounding savanna, where a dense 50 cm hardpan of calcium carbonate at 50 cm depth (Sinclair 1979) prevents any woody species to root in deeper soil layers. As a result, plant species that, due to higher water requirements, are unable to survive the dry season in most of the savannah, might potentially grow on kopjes. Second, it has been suggested that the vegetation on kopjes escapes fires, since the bare rocks act as a fire break (Hoeck 1975). Third, kopjes provide shade and oversight opportunities for large predator species, making them a popular resting site for these species (Hopcraft et al. 2005; Durant et al. 2010). As a result, kopjes might be avoided by many herbivorous mammal species as a too risky place. We thus expect that both spatial variation in rainfall and the presence of kopjes contribute to community turnover in savannahs, and consequently to overall biodiversity.

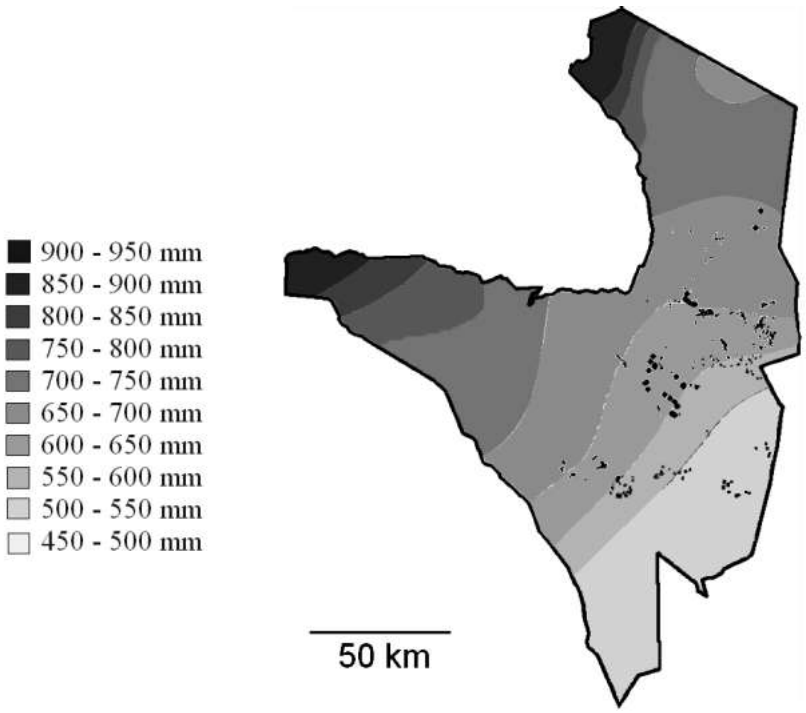


**Figure 5.2.** Map of Serengeti National Park (SNP) with the red dots representing the kopjes investigated during this study. The brown polygons represent all known kopjes in SNP. The background colours represent the rainfall gradient in SNP, with average annual amount of rainfall ranging from 450 mm per year to over 900 mm per year.

To test these ideas, we investigated 24 kopjes and 24 adjacent matrix plots positioned along a rainfall gradient in Serengeti National Park (SNP) and characterized tree and mammal communities. Additionally, we measured several functional traits of trees (specific leaf area, leaf nitrogen content, spinesence) and collated other tree (fine/broad leaved) and mammal (size, diet) traits from the literature. With these data, we investigated the following questions: (i) how do tree and mammal richness respond to spatial variation in rainfall and habitat type (kopje vs. adjacent site); (ii) are tree and mammal community responses to variation in rainfall and habitat type reflected by trait responses and (iii) to what extent do tree and mammal responses to variation in rainfall and habitat type interact with each other?

# 5.3 Methods

## 5.3.1 Study site

Serengeti National Park (SNP) is a large protected area in northwestern Tanzania (34° to 36° E and 1° to 3° 30' S). There is a strong rainfall gradient within SNP, with mean annual amount of rainfall ranging from less than 500 mm in the southeast to over 900 mm in the northwest (Fig. 5.2). This rainfall gradient coincides with transitions in main habitat types, with treeless plains in the southeast, savanna vegetation dominating more in the centre of SNP and woodlands becoming more common in the northwest (Reed et al. 2009). At smaller scales, rivers, termite mounds and rocky outcrops add to the habitat heterogeneity in SNP.

## 5.3.2 Site and plot selection, vegetation and sampling

Within SNP, over a rainfall gradient (from ~650 mm annual rainfall to ~750 mm), we visited 24 'kopje plots' and 24 adjacent, equal-sized adjacent plots (hereafter: matrix plots) in October-December 2010, to characterize the tree communities (Fig. 5.2). Kopjes were at least 62.5 meters away from each other, but usually much further, with a mean nearest neighbor distance of 1433 meter and the furthest kopjes being over 67 km away from each other (Fig. 5.2). We sampled vegetation within the smallest rectangle ('plot') that could be drawn around a kopje. The area of these plots ranged from 285 $m^2$ to 2000 $m^2$, with an average size of 588 $m^2$. Between 50 and 100 meters away from the kopje plot, in a random direction, we visited a matrix plot, which had the same size and shape as the associated kopje plot. We made sure that the matrix plot was not within 50 meters from another kopje, so that we could assume that the vegetation characterized in these matrix plots was representative for the savanna vegetation. In each plot, we identified all individual trees to species level. Identification was performed using Van Wyk & Van Wyk (1997), local experts and the Seronera Research Station herbarium collection. Additionally, we estimated the height of each individual to a precision of 0.5 m. Two times, with an interval of approximately one month, we walked in a zig-zag way along transects spaced 2m away from each other until we covered the whole plot. We identified and counted all droplets belonging to all mammal species with a body size over 2 kg. Previous studies have demonstrated the accuracy of dropping counts as a measure for mammal richness (Cromsigt et al. 2009). Identification of droplets was done using Stuart & Stuart (2000).

## 5.3.3 Collection of tree and mammal trait data

In each plot, we measured 3 traits for each observed species: Specific Leaf Area (SLA), leaf Nitrogen content (N) and spine density. SLA was calculated by dividing the area of a fresh leaf by its dry weight. It is a measure of leaf thickness, with high SLA values corresponding to thin leaves and low SLA values corresponding thick leaves. SLA usually correlates positively with relative growth rate, but negatively with leaf longevity (Reich et al. 1997; Weiher et al. 1999; Westoby et al. 2002). A photograph of at least 10 fresh, green leaves, together with a reference square with a known area was taken in the field. Because many leaves tended to fold during sunny and hot weather, we picked the leaves and placed them in a shaded location for at least 15 minutes before photographing. The software program ImageJ (Abramoff et al. 2004) was used to calculate total area of photographed leaves in $cm^2$. The leaves were dried, after which weight was measured. These dry weights were used to calculate SLA (in $cm^2\ g^{-1}$), by dividing total leaf area by total dry weight. Spine density, which acts

as a defense against browsers (Cooper and Owen-Smith 1986), was measured by taking at least 5 branches from between 10 and 20 cm and counting the number of spines per branch length. For measurements of leaf N content, we collected bulk samples of fresh leaves of each species. These were dried and ground with a bullet grinder. We measured leaf N content for a subset of the samples (11 %) using a Carlo-Erba NA 1500 (Carlo-Erba, Milan, Italy). Leaf N concentrations of the other samples were estimated using a spectrophotometer (Bruker MPA NIR) and an associated calibration line, partially based on the chemically measured samples from this study and partially based on the samples measured in a previous study (Van der Plas et al. 2013). Leaf N content is usually correlated with relative growth rate (Reich et al. 1997) and additionally correlates with a species' attractiveness to browsers (Cooper and Owen-Smith 1985; Van der Plas et al. 2013). Each tree species observed in this study was either classified as fine-leaved, if its leaves were compound and bipinnate, or broad-leaved, if its leaves were not bipinnate. Previous work suggests that fine-leaved savanna tree species are generally more fire resistant than broad-leaved tree species (Sharam et al. 2006). Mammal trait data was collated from Kingdon (2003), in which we looked up body size (in kg) and diet choice (predator, omnivorous, browser, grazer or mixed herbivorous feeder).

### 5.3.4 Calculating average trait values across plots

The measured trait values were used to calculate the abundance-weighted trait value on each plot: $PAWTA_i = \sum_{n=1}^{S} a_n t_n$, in which $PAWTA_i$ is the Abundance-Weighted Trait Average of the i-th Plot, S the species richness of the i-th plot, $a_n$ the abundance of the n-th species and $t_n$ the trait value of the n-th species. Sometimes species values were missing; in these cases the average trait value of the species across all plots was used to calculate PAWTA.

### 5.3.5 Statistical analyses

We investigated how tree species richness, tree density, tree functional traits, mammal species richness and log abundance of each species responded to rainfall and habitat type (surroundings site vs. kopje). We performed general linear mixed models (GLMMs), with rainfall, habitat type and their interaction as fixed factors, and site as random factor. We than ran a procedure based on maximum likelyhood to select the best fitting model with lowest AIC value including significant predictor variables. This was done using the lme function from the nlme package (Pinheiro et al. 2012) in R-2.13.1 (R Development Core Team 2011).

## 5.4 Results

### 5.4.1 Tree community richness and composition patterns

In total, we identified 62 tree species (Table 5.2), of which 19 rare ones could only be identified to morphospecies. Of all species found, 51 were observed in kopje plots, while only 21 species were found in surroundings plots. 19 tree species were significantly more abundant in kopje plots than in matrix plots (Fig. 5.3; Table 5.1), while 2 tree species (*Acacia senegal* and *A. tortilis*) were significantly more common in savannah matrix (Fig. 5.3; Table 5.1). Kopje sites contained significantly more tree species than matrix plots: on average, 12.4 species were found on kopjes, while an average of only 3.4 species was found on matrix plots (Fig. 5.3, Table 5.1). Additionally, kopje plots contained many more individual trees than surroundings plots, with on average 98.7 trees found on kopjes and only 23.9 trees found in matrix plots (Fig. 5.3, Table 5.1). Both tree species richness and tree abundance also responded positively to annual rainfall (Table 5.1). Tree abundance increased more steeply with rainfall in matrix plots than in kopje plots.

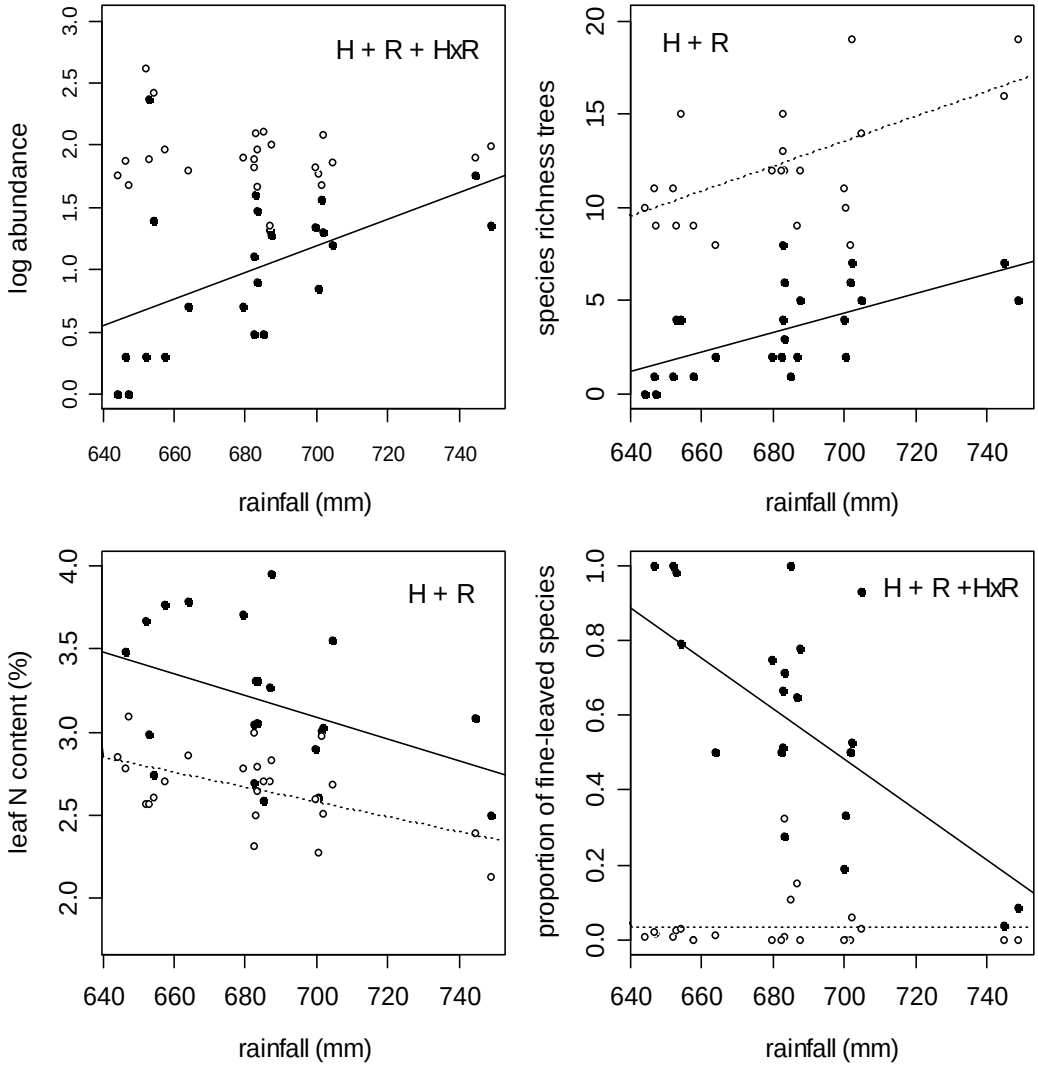


**Figure 5.3.** Responses of tree abundance, species richness, community-average leaf nitrogen content and proportion of fine leaved individuals to annual amount of rainfall and habitat type. Red dots represent kopje plots, while blue dots represent control plots.

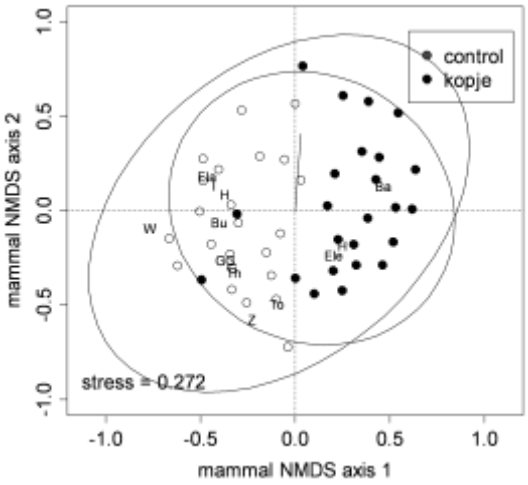


**Figure 5.4.** Biplot of the first two NMDS axes of tree (left) and mammal (right) communities. Control/surroundings plots are given in white, kopje plots in grey. Ellipses around the dots represent the two dimensional confidence intervals (±2SD) of average NMDS values of both kopje and control plots. Tree species that were significantly more abundant in either one of the habitats are shown in NMDS space (left graph) with abbreviations: species more abundant in control sites: AS = *Acacia Senegal*, AT = *Acacia tortilis*; species more abundant on kopjes: A = *Allophyllus rubifolius*, B = *Boscia angustifolia*, C = *Croton dichogamus*, CA = *Commiphora africana*, CAe = *Cassine aethiopica*, CO = *Cordia ovalis*, CT = *Commpihora trothae*, CTo = *Capparis tomentosa*, E = *Euclea natalensis*, F = *Ficus glumosa*, G = *Grewia bicolor*, GF = *Grewia fallax*, I = *Iboza riparia*, P = *Pappea capensis*, PA = *Pavetta assimilis*, R = *Rhus natalensis*, T = *Teclea trichocarpa*, TF = *Turraea fischeri* and Z = *Zizyphus mucronata*. Mammal species that were significantly more abundant in either one of the habitats are shown in the right graph in NMDS space: H (hyena): most abundant in control plots; T (topi), GG (Grant's gazelle) and TG (Thompson's gazelle): most abundant in control sites.

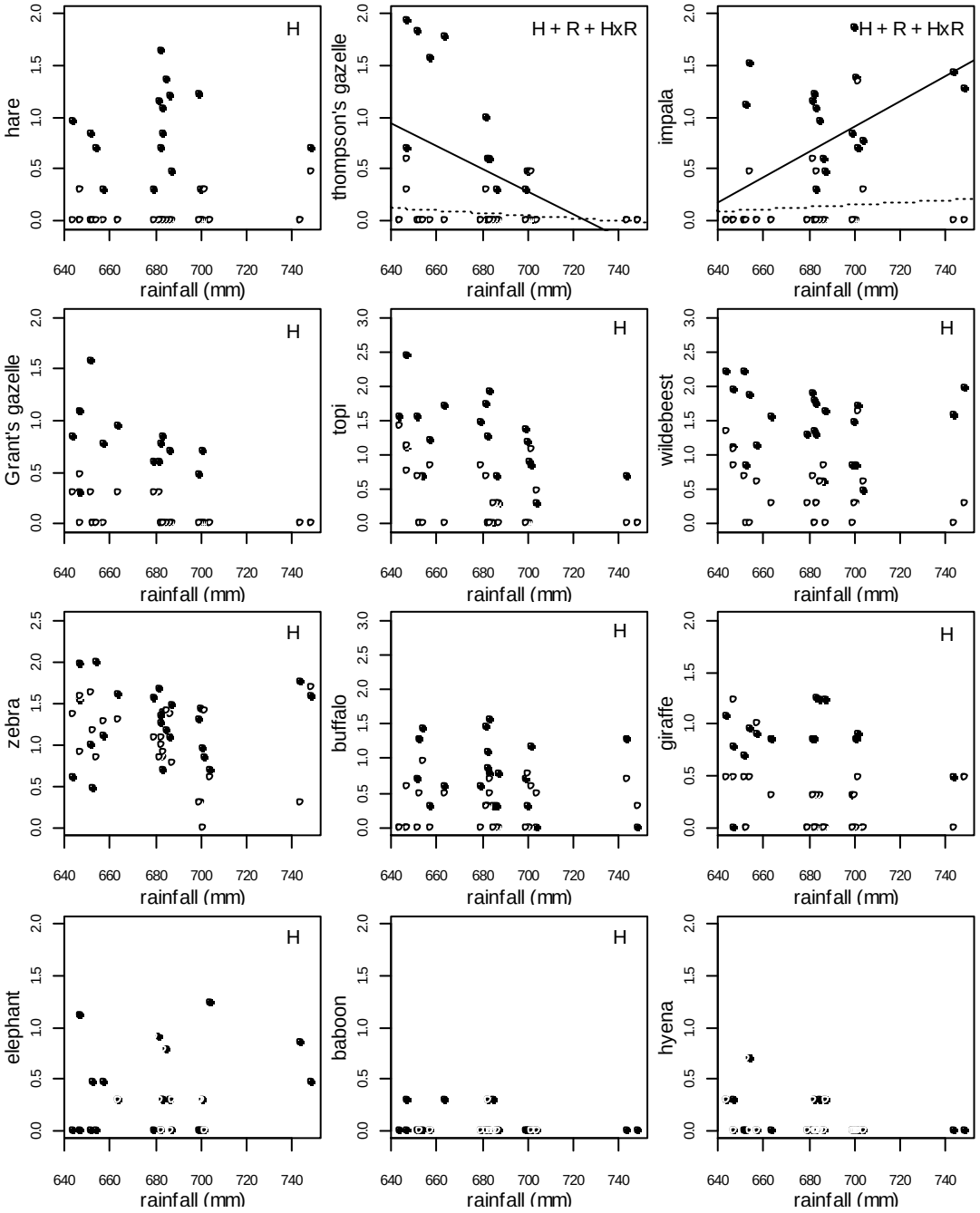


**Figure 5.5.** Responses of the 12 most frequently observed mammal species to annual amount in rainfall and to habitat type. Red dots represent kopje plots, while blue dots represent control plots.

**Table 5.1.** The significant variables of selected (with an ML selection procedure) models explaining richness, abundance, functional and composition patterns of tree and mammal communities. Only mammal species that were observed in at least 15 plots are included in this table. Between the brackets the *t* and *P* value of the significant predictor variables are given. ns = no significant predictor variables. Abbreviations: vs = very small (< 15 kg), s = small (> 15, < 60 kg), m = medium-sized (> 60, < 250 kg), l = large (> 250 kg).

| Response variable | Variables in final model with *F* and *P* value |
|---|---|
| **Trees** | |
| Abundance (log) | Rainfall ($t$ = 3.396, $P$ = 0.0026), kopje ($t$ = 2.991, $P$ = 0.0067), rainfall x kopje ($t$ = -2.692, $P$ = 0.0133) |
| Species richness | Rainfall ($t$ = 4.124, $P$ < 0.0001), kopje ($t$ = 12.324, $P$ < 0.0001) |
| N content | Rainfall ($t$ = -2.839, $P$ = 0.0096), kopje ($t$ = -7.168, $P$ < 0.0001) |
| Spine density | Rainfall ($t$ = -2.555, $P$ = 0.0180), kopje ($t$ = -6.901, $P$ < 0.0001) |
| SLA | Kopje ($t$ = 4.460, $P$ < 0.0001) |
| Fine-leaved | Rainfall ($t$ = -4.214, $P$ = 0.0004), kopje ($t$ = -3.471, $P$ = 0.0024), rainfall x kopje ($t$ = 3.100, $P$ = 0.0056) |
| **Mammal comm.** | |
| Total species richness | Kopje ($t$ = -2.235`, $P$ = 0.0354) |
| **Mammal abund.** | |
| Hare (vs) | Kopje ($t$ = -5.381, $P$ < 0.001) |
| Thomson's gazelle (s) | Rainfall (t = -2.656, P = 0.018), kopje ($t$ = -2.429, $P$ = 0.024), rainfall x kopje ($t$ = 2.288, $P$ = 0.032) |
| Impala (s) | Rainfall (t = 3.000, P = 0.009), kopje ($t$ = 2.474, $P$ = 0.022), rainfall x kopje ($t$ = -2.676, $P$ = 0.014) |
| Grant's gazelle (s) | Kopje ($t$ = -4.463, $P$ < 0.001) |
| Topi (m) | Kopje ($t$ = -4.760, $P$ < 0.001) |
| Blue wildebeest (m) | Kopje ($t$ = -7.502, $P$ < 0.001) |
| Plains zebra (m) | Kopje ($t$ = -2.314, $P$ = 0.030) |
| African buffalo (l) | Kopje ($t$ = -3.490, $P$ = 0.002) |
| Giraffe (l) | Kopje ($t$ = -3.291, $P$ = 0.003) |
| African elephant (l) | Kopje ($t$ = 3.439, $P$ = 0.002) |
| Olive baboon | Kopje ($t$ = 4.690, $P$ < 0.001) |
| Spotted hyena | ns |

Functionally, there were differences in tree traits between kopje plots and matrix plots. Trees growing on kopjes had higher SLA, lower leaf N content (Fig. 5.3) and fewer spines than trees growing in matrix plots (Table 5.1). Additionally, there was a significantly higher proportion of fine-leaved trees in matrix plots (57.9%) than on kopjes (3.3%), and within matrix plots, fine leaved species were relatively more common in the low rainfall areas (Fig. 5.3, Table 5.1). Leaf N content and tree spinesence were lower in sites with high rainfall (Table 5.1).

### 5.4.2 Mammal community richness and abundance patterns

In total, droppings of 20 mammal species were found (Table 5.3). Of these, 18 species were found in at least one matrix plot, with only rock hyrax and steinbuck being absent there. Also 18 species were found in at least one kopje plot: only kongoni and reedbuck were not observed on kopjes. Three mammal species (rock hyrax (GLLM: $T$ = 2.240, $P$ = 0.035), elephant and olive baboon), all either very small or very large, were significantly more common on kopjes. Ten other species, more intermediate in body size, were most common in matrix plots: eland (which also responded negatively to rainfall, GLMM, rainfall: $T$ = 2.172, $P$ = 0.046; kopje: $T$ = -3.878, $P$ < 0.001), hare, Thompson's

gazelle, impala, Grant's gazelle, topi, wildebeest, zebra, buffalo and giraffe (Table 5.1). Richness was significantly higher in matrix plots (average of 8.8 species) than on kopjes (average of 6.8 species) (Table 5.1). Of these, the abundance of Thompson's and gazelle and impala also responded to variation in annual rainfall: both species were, within matrix sites, more common in lower rainfall areas (Table 5.1).

**Table 5.2**. Tree species identified during this study. Column explanations: S: the number of surroundings plots in which the given species occurred, with bold and underlined values indicating whether the species was significantly more common in the given habitat type; K: the number of kopje plots in which the given species occurred, with bold and underlined values indicating whether the species was a significant kopje indicator species. Rain: the relationship of the species abundance with rain: positive (+), negative (-) or rainxhabitat type interaction effect (x). N content, SLA, SD (spine density) and F (fine-leaved): the average trait values measured for the given species.

| Name | Family | S | K | rain | N content (%) | SLA ($cm^2g^{-1}$) | SD | F |
|---|---|---|---|---|---|---|---|---|
| *Acacia brevispica* | Mimosacecae | 0 | 2 | | 3.478 | 115.9 | 9.25 | Yes |
| *Acacia drepanolobium* | Mimosacecae | 2 | 0 | | 3.489 | 55.05 | 26.0 | Yes |
| *Acacia gerrardi* | Mimosacecae | 1 | 0 | | | | | Yes |
| *Acacia hockii* | Mimosacecae | 2 | 0 | + | | 78.71 | 19.3 | Yes |
| *Acacia nilotica* | Mimosacecae | 0 | 2 | | 2.693 | 70.41 | 10.3 | Yes |
| *Acacia robusta* | Mimosacecae | 8 | 2 | | 2.590 | 57.77 | 7.83 | Yes |
| *Acacia senegal* | Mimosacecae | **10** | 5 | | 4.26 | 75.74 | 28.0 | Yes |
| *Acacia tortilis* | Mimosacecae | **14** | 6 | | 3.372 | 69.75 | 8.75 | Yes |
| *Acacia xanthophloea* | Mimosacecae | 1 | 2 | | 3.667 | 51.15 | 23.7 | Yes |
| *Albizia harveyi* | Mimosacecae | 11 | 5 | | 2.831 | 52.31 | 0.31 | Yes |
| *Allophylus rubifolius* | Sapindaceae | 0 | **18** | | 2.189 | 69.51 | 0.00 | No |
| *Aloe volkensii* | Liliaceae | 0 | 1 | | 2.105 | | 16.0 | No |
| *Balanites aegyptica* | Balanitaceae | 2 | 1 | | 2.815 | 38.34 | 8.27 | No |
| *Boscia angustifolia* | Capparaceae | 0 | **10** | | 2.230 | 61.47 | 8.09 | No |
| *Boswelia neglecta* | Burseraceae | 1 | 4 | + | 1.936 | 56.46 | 0.00 | No |
| *Capparis tomentosa* | Capparaceae | 0 | **3** | | 3.253 | 49.44 | 15.7 | No |
| *Cassine aethiopica* | Celastraceae | 0 | **4** | | 2.294 | 54.20 | 1.25 | No |
| *Combretum molle* | Combretaceae | 2 | 2 | + | 2.010 | 48.94 | 0.00 | No |
| *Commiphora africana* | Burseraceae | 0 | **3** | | 2.364 | 82.88 | 3.36 | No |
| *Commiphora trothae* | Burseraceae | 13 | **21** | | 2.358 | 78.10 | 6.06 | No |
| *Cordia ovalis* | Boraginaceae | 0 | **20** | | 2.847 | 72.40 | 0.00 | No |
| *Croton dichogamus* | Euphorbiaceae | 0 | **5** | x | 2.584 | 71.56 | 0.00 | No |
| *Dicrostachys cinerea* | Mimosacecae | 0 | 1 | | 3.298 | 86.03 | 3.00 | Yes |
| *Euclea natalensis* | Ebenaceae | 0 | **8** | x | 1.977 | 47.18 | 0.00 | No |
| *Euphorbia ingens* | Euphorbiaceae | 0 | 1 | | | | 0.00 | No |
| *Ficus glumosa* | Moraceae | 0 | **16** | | 1.928 | 62.80 | 0.00 | No |
| *Ficus ingens* | Moraceae | 0 | 1 | + | 2.375 | 53.17 | 0.00 | No |
| *Grewia bicolor* | Tiliaceae | 0 | **23** | | 2.683 | 72.07 | 0.00 | No |
| *Grewia fallax* | Tiliaceae | 1 | **9** | | 2.941 | 59.24 | 0.00 | No |
| *Grewia tembensis* | Tiliaceae | 2 | 4 | | 3.011 | 81.07 | 0.00 | No |
| *Iboza riparia* | Lamiaceae | 0 | **12** | x | 3.071 | 110.9 | 0.00 | No |
| *Lippia javanica* | Verbenaceae | 1 | 7 | | 3.122 | 60.81 | 0.00 | No |
| *Maerua edulis* | Capparaceae | 2 | 0 | | | 30.02 | 0.00 | No |
| *Maerua triphylla* | Capparaceae | 0 | 2 | | 4.508 | 93.07 | 0.00 | No |
| *Ormocarpum trichocarpum* | Mimosacecae | 6 | 5 | + | 3.537 | 67.21 | 0.00 | No |
| *Pappea capensis* | Sapindaceae | 0 | **11** | x | 2.097 | 59.77 | 0.00 | No |
| *Pavetta assimilis* | Rubiaceae | 0 | **16** | | 2.599 | 68.83 | 0.00 | No |
| *Phyllanthus sepialis* | Euphorbiaceae | 0 | 4 | | 3.116 | 105.3 | 0.00 | No |
| *Rhus natalensis* | Anacardiaceae | 0 | **6** | x | 1.902 | 57.81 | 0.00 | No |
| *Solanum incanum* | Solanaceae | 1 | 0 | | 3.537 | 64.53 | 0.00 | No |
| *Teclea trichocarpa* | Rutaceae | 0 | **7** | | 4.586 | 56.76 | 0.757 | No |
| *Turraea fischeri* | Meliaceae | 1 | **21** | | 2.749 | 74.43 | 0.00 | No |
| *Ziziphus mucronata* | Rhamnaceae | 0 | **5** | x | 3.194 | 105.0 | 13.97 | No |

**Table 5.3.** The identified mammal species of this study. Column explanations: s: the number of surroundings plots in which the given species occurred, with bold and underlined values indicating whether the species was a significant surroundings plot indicator species; k: the number of kopje plots in which the given species occurred, with bold and underlined values indicating whether the species was a significant kopje indicator species. R: whether a species is ruminant (yes) or not (no).

| common name | scientific name | s | k | diet | size (kg) | r |
|---|---|---|---|---|---|---|
| African buffalo | *Syncerus caffer* | 18 | 13 | grazer | 550.00 | yes |
| wildebeest | *Connachaetes taurinus* | 23 | 17 | grazer | 213.75 | yes |
| reedbuck | *Redunca redunca* | 1 | 0 | grazer | 47.00 | yes |
| eland | *Taurotragus oryx* | 12 | 2 | mixed feeder | 560.50 | yes |
| elephant | *Loxodonta africana* | 12 | 22 | mixed feeder | 2150.00 | no |
| giraffe | *Giraffa camelopardalis* | 17 | 12 | browser | 1340.00 | yes |
| Grant's gazelle | *Nanger granti* | **12** | 6 | mixed feeder | 61.63 | yes |
| hare | *Lepus spp.* | 18 | 2 | mixed feeder | 2.27 | no |
| impala | *Aepyceros melampus* | 16 | 5 | mixed feeder | 56.25 | yes |
| jackal | *Canis spp.* | 4 | 3 | omnivore | 9.97 | |
| Kirk's dik-dik | *Madoqua kirkii* | 4 | 0 | mixed feeder | 5.50 | yes |
| klipspringer | *Oreotragus oreotragus* | 3 | 8 | mixed feeder | 13.00 | yes |
| kongoni | *Alcelaphus buselaphus* | 2 | 0 | grazer | 161.00 | yes |
| baboon | *Papio anubis* | 4 | 15 | omnivore | 28.25 | |
| zebra | *Equus quagga* | 23 | 23 | grazer | 241.75 | no |
| rock hyrax | *Procavia capensis* | 0 | 7 | mixed feeder | 3.15 | no |
| spotted hyena | *Crocuta crocuta* | 6 | **9** | carnivore | 65.00 | |
| steinbuck | *Raphicerus campestris* | 2 | 1 | mixed feeder | 11.50 | yes |
| Thomson's gazelle | *Gazella rufifrons* | **12** | 3 | mixed feeder | 23.75 | yes |
| topi | *Damaliscus korrigum* | **18** | 11 | grazer | 126.25 | yes |

## 5.5 Discussion

Abundance and diversity of trees increased with rainfall. Other studies in African savannas have shown similar patterns, with generally highest tree abundances and diversity in high rainfall areas (Scholes et al. 2002; 2004; Eshete et al. 2011). Rainfall is one of the most limiting factors for plant growth in savannahs (Kraaij and Ward 2006) and in wetter areas, trees are suggested to get a competitive advantage over grasses (Sankaran et al. 2004). Additionally, the driest sites in SNP are characterized by a shallow, impermeable hardpan (Sinclair 1979), that roots cannot penetrate. Therefore, if any, only tree species with shallow roots can live in these areas, explaining why tree abundances were so much higher in wetter sites.

Abundance and diversity of trees did not only increase with rainfall, but responded strongly to the presence/absence of kopjes. On kopjes, on average approximately 4.1 times as many individuals and 3.6 times as many species of trees were found than in matrix plots (see for illustrations Fig. 5.1 and for a graph, Fig. 5.2). A reason why tree abundances and richness are so much higher on kopjes than in the savanna matrix might be because the kopjes were also characterized by lower browser densities. Several browser / mixed feeders species (Thomson's and Grant's gazelle, impala, giraffe and eland) were less abundant on kopjes than in the matrix savanna. A reduced browsing intensity could imply that many more tree seedlings on kopjes survive to a sapling stage or to adult trees than in the savanna matrix, resulting in much higher abundances. Additionally, with a low abundance of browsers, not all tree species need to be tolerant to browsing. Supporting this idea is the fact that kopje tree species had on average fewer spines than trees from the surroundings. However, contrary to this interpretation, two other (partially) browsing mammal species, elephant and rock hyrax, were more common on kopjes. Therefore, based on our limited amount of data on herbivore distributions, it is hard to fully

compare the browsing pressure between kopje and surroundings sites. Another possible reason why tree abundances and richness were higher on kopjes, may be because kopjes supposedly burn less frequently than vegetation of the savanna matrix (Poelchau & Mistry 2006). Fires strongly suppress the recruitment of woody species in savannas (Higgins et al. 2000), and of broad-leaved species in particular (Sharam et al. 2006). In line with this, kopjes contained relatively many more broad-leaved species, with all 15 kopje indicator tree species being broad-leaved, and additionally, all tree species that were significantly most abundant on kopjes were fine-leaved. Another possible reason why kopjes contain more tree species than matrix plots, might be the suggested high water availability in the cracks between rocks (Poelchau & Mistry 2006) and the possibility of water penetration to deeper layers. As a result, kopjes could potentially harbor many drought-intolerant species. In general, high drought tolerance correlates with low SLA values (Westoby et al. 2002) and one would thus expect that trees growing on kopjes have relatively high SLA. This was indeed the case. However, we should emphasize that for the fine-leaved species growing mostly in the surroundings, SLA was difficult to measure due to the folding of leaflets when temperatures were high. Although leaves used for SLA measurements were put in the shade at least 15 minutes prior to measurements, measurements might still be biased towards lower values for fine-leaved species.

Mammal richness did not respond to spatial variation in rainfall, but abundances of some individual species did. Droppings of Thompson's gazelle and impala were most abundant in low rainfall areas. However, we should keep in mind that mammal droplings were counted in a period that characterized the shift from the long dry season to the short wet season (Sinclair et al. 2008). Large scale distributions of many herbivores usually shift dramatically over seasons due to migrations (Maddock 1979; Holdo et al. 2009) and therefore one should be careful with interpreting these observations. One potential reason why Thompson's gazelle and impala were most abundant in lower rainfall areas, is that these sites are also characterized by relatively high foliage quality in the wetter periods of the year (Holdo et al. 2009). A question remaining, however, is whether these species are also most abundant in drier areas during other parts of the year. Thompson's gazelle has been shown to be migratory and is more abundant in wetter parts of the Serengeti in the dry season, while being more abundant in relatively low rainfall areas in the wet season (Maddock 1979). Impalas are not migratory, and therefore for this species the abundance patterns are more likely to be consistent over seasons.

At smaller scales, mammals also responded to habitat type. Richness of mammals was lower on kopjes than in surrounding savanna. Also abundances of many mammal herbivore species, including browsers, were lower on kopjes. This raises the question why on a habitat with so much food availability (high tree density), so few browsers and other herbivores were present. A possible explanation is that herbivores avoid predators associated with kopjes. Due to their high elevation when compared to the surrounding, kopjes form excellent lookout points and resting locations for large predators, such as lions, hyenas and leopards. In line with this, there was a (albeit non-significant) trend for more droppings of spotted hyena on kopjes, while other studies have shown that also lions, leopards and other predators are positively associated with kopjes (Hopcraft et al. 2005; Durant et al. 2010). As such, kopjes likely function as a 'landscape of fear' (Laundre et al. 2001) for mammalian prey species. Predation risk is usually highest for small and medium sized herbivores (Sinclair et al. 2003; Hopcraft et al. 2010), while megaherbivores like elephants are less likely to suffer from predation. In line with this, some small and medium-sized herbivores (e.g. Thompson's gazelle, Grant's gazelle, topi, zebra and wildebeest) were less abundant in kopje sites than in the surrounding matrix, while elephants were more abundant on kopjes. A second possible reason why some herbivores avoid kopjes, is because kopjes do not contain much food (i.e. grass) for pure grazer species, such as topi, zebra, wildebeest and buffalo (Poelchau & Mistry 2006).

Although most herbivore species were less abundant on kopjes, there are some small sized herbivorous species that do not follow the main trend. Rock hyrax was found to be positively associated with kopjes, while some other small herbivore species (klipspringer, steenbuck) also tended to be associated with kopjes, although these species were too rare allow robust statistical analyses. Other literature suggests that some of these species are in fact associated with kopjes

(Kingdon 2003; Baskerville et al. 2011). We suggest that while most small or medium sized herbivores (body weight between 20 and 200 kg) avoid kopjes due to the presence of predators, some even smaller-sized species (body size below 20 kg) are confined to kopjes, because their very small bodies allow them to escape from large predators by hiding between rock cracks. Additionally, the largest herbivore (elephant) did not avoid kopjes either, possibly because with its large body size, it hardly suffers predation. The high density of food (trees) on kopjes makes these habitats even very attractive for these very small and very large browsing species.

## 5.6 Conclusions

Both tree and mammal communities responded strongly to gradients in rainfall and the presence of kopjes. Most species and individuals of trees were found in high rainfall sites and on kopjes, with trait shifts suggesting that these shifts were due to higher water availability and lower herbivore pressure in wetter areas and on kopjes, and additionally to lower fire frequency on kopjes. Probably as a result of the high predation risk found there, abundances of many herbivore species were lower on kopjes. Only the smallest and largest herbivore species were more abundant on kopjes, possibly because these species were respectively small enough to hide from predators or too large to be killed by predators. Our study shows how habitat heterogeneity affects community assembly of trees and herbivores in savannas, with the strong possibility of direct and indirect trophic interactive effects between these and other groups explaining their (lack of) affinity to kopjes.

## 5.7 Acknowledgements

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# Section II: Ecosystem engineering in community assembly

# Chapter 6: Functional traits of trees on and off termite mounds: understanding the origin of biotically-driven heterogeneity in savannahs.

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## 6.1 Abstract

**Questions:** In African savannahs, *Macrotermes* termites contribute to small-scale heterogeneity by constructing large mounds. Operating as islands of high nutrient and water availability and low fire frequencies, these mounds support distinct, diverse communities of trees which have been shown to be highly attractive for browsers. However, the distinct traits of tree species on termite mounds have hardly been studied, even though this may help to understand the processes determining (1) their characteristic community structure and (2) attractiveness for browsers. Here, we compare functional trait and browser preference values between tree species on and off termite mounds.
**Location:** Hluhluwe-iMfolozi Park, Kwazulu-Natal, South-Africa
**Methods:** We recorded tree community compositions for 16 large *Macrotermes natalensis* mound and 16 control plots of 100 $m^2$ each, in a paired design. For each observed tree species we measured 22 traits, related to water and nutrient use, fire tolerance, light competition and anti-herbivore defense, and we compared average trait values between mound and control communities. Furthermore, we investigated the feeding preferences of ungulate browsers for the most common tree species and how this was linked to their associated traits.
**Results:** Termite mounds supported tree communities that were distinct from their surrounding savannah vegetation. Mounds hosted more evergreen and less leguminous tree species than control communities, and the dominant species were less mechanically defended, less nutritious, had larger leaves and lower wood density than the species dominating control plots. Browsers preferred leguminous tree species with high leaf nitrogen and phosphorous content, which were relatively rare on termite mounds.
**Conclusions:** Overall, we conclude that termite mounds in this savanna form small refugia for tree species which seem less adapted to fire (more evergreens), low nutrient availability (less nitrogen fixers) and water stress (larger leaf sizes) than typical savannah trees. Surprisingly, despite their reputation as browsing hotspots, the tree species dominating them are less nutritious and less preferred by browsers than tree species from the surrounding savanna, which may be explained by the relatively nutrient rich nature of this savannah or intraspecific trait differences.

## 6.2 Introduction

Savannahs belong to the most species rich ecosystems on earth (Mittermeier et al. 1998), for which their high spatial heterogeneity plays an important role (Scholes 1990). This heterogeneity is found at several spatial scales, with different abiotic and biotic processes creating heterogeneity at each scale (Scholes 1990; Gilson 1994; Cromsigt 2006). At large spatial scales, rainfall patterns can drive habitat heterogeneity (e.g. Sinclair et al. 2008). At intermediate scales, fire, geological and soil factors become more important (Scholes 1990; Higgins et al. 2000), while at the smallest scales, heterogeneity is mainly driven by biotic agents, such as mammalian herbivores (Cromsigt 2008; Waldram et al. 2008) or termites (Moe et al. 2009; Okullo & Moe 2012; Gosling et al. 2012). In small reserves, where large landscape-level gradients are often less important, the biotic drivers of local scale heterogeneity are most essential in creating and maintaining a high biodiversity (Cromsigt 2008).

Mound building termites, such as *Macrotermes spp*, are key drivers of heterogeneity at these local scales (Dangerfield et al. 1998; Sileshi et al. 2010). It has been shown that their mounds constitute only approximately 5% of the savanna landscape, but with their high tree densities, they highly contribute to the small-scale variation in woody vegetation cover (Moe et al. 2009; Levick et al. 2010). In addition, the tree communities found on mounds are often very diverse, hosting many species that are not found elsewhere in the savannah (Traoré et al. 2008; Moe et al. 2009; Okullo & Moe 2012). These tree species supply popular food for herbivores, with megaherbivores preferentially browsing mound trees (Holdo & McDowell 2004; Loveridge & Moe 2004; Levick et al. 2010). However, the determinants of the altered tree community composition on termite mounds, such as modified nutrient, water and light availability and fire regimes have remained poorly studied until now. Furthermore, we do not understand why the trees found on termite mounds are so attractive for ungulate browsers. Exploration of the functional traits of the dominant tree species may shed light in these determinants. Therefore, here we investigate how traits related to stress tolerance, competition for light and plant mineral nutrition differ between tree species dominating on and off *Macrotermes natalensis* mounds in an African savannah. Furthermore, we investigate the relationships between these traits and attractiveness for browsers.

Several abiotic and biotic factors have been suggested to play an important role in the local community assembly of savannah trees: drought, fire, soil fertility and competition for light (Walter 1971; Frost et al. 1986; Scholes & Walker 1993; Bond & Wilgen 1996; Cramer et al. 2010). All of these factors can be locally modified by *Macrotermes* termites because of the construction of their mounds (Dangerfield et al. 1998) and removal of litter and plants nearby. The soil used for the construction of mounds often comes from deeper soil layers (Holt & Lepage 2000) and has therefore a higher clay content and elevated concentrations of several cations, inorganic carbon, and extractable nitrate and ammonia (Holt & Lepage 2000; Okullo & Moe 2012, Gosling et al. 2012). The finer soil texture causes a higher water potential of the mound soil (Konaté et al. 1999). In addition, the construction of subterranean feeding galleries and tunnels increases soil infiltration rates and improves soil water transmission properties in the surrounding of the mounds, thereby furthermore increasing soil water availability (Holt & Lepage 2000). Also, termite mound vegetation is suggested to be less affected by fire than the surrounding savannah (Dangerfield et al. 1998; Moe et al. 2009). The higher tree densities that may result from higher water availability and less fire impact (Loveridge & Moe 2004; Traoré et al. 2008; Moe et al. 2009; Levick et al. 2010), may cause stronger competition for light among these trees on mounds.

The effects of *Macrotermes* termites on plant resource availability, competition and fire regimes are expected to be reflected in the traits of tree species that preferentially occur on mounds. These same traits can also be important in explaining why browsers preferentially feed on trees dominating on termite mounds: most browser species do not feed randomly, but prefer foliage with high nutrient contents and low chemical and mechanical defences (Cooper & Owen-Smith 1985, 1986; Owen-Smith & Cooper 1987; Emslie 1999).

Therefore, in this study, we use a trait-based approach (McGill et al. 2006) to unravel how termite mounds affect tree community structure and browsing intensity. We compared values of several plant traits related to drought, fire, and herbivory resistance and attractiveness, light competition and nutrient limitations between woody species dominating termite mounds and species dominating the surrounding savannah. Furthermore, we studied the feeding preferences of ungulate browsers and investigated how these preferences are related to plant species traits. This may help to understand the unique ecological role of termite mounds as key determinants of savannah heterogeneity, specifically in forming browsing hotspots for large herbivores.

# 6.3 Methods

## 6.3.1 Study area

Field work was carried out in Hluhluwe-iMfolozi Park (HiP), an 89,665 ha mesic savannah nature reserve in Kwazulu-Natal, South Africa (S6.1). Altitude ranges from 40 to 750 m above sea level. Most areas in the park burn frequently, with a mean fire return period of 3.8 years (Balfour &

Howison 2002). Vegetation types vary from grasslands, savannahs, broad leaved thickets, to upland forest. Broad leaved woodland communities are dominated by *Euclea divinorum* or *Spirostachys africana*, but more than half of HiP consists of savannah dominated by *Acacia* spp., with varying amounts of woody coverage (Whateley & Porter 1983). HiP contains a rich assemblage of browsing ungulate species, with the more common ones, in decreasing order of body size, being: African elephant (*Loxodonta africana*), black rhino (*Diceros bicornis minor*), giraffe (*Giraffa camelopardalis*), kudu (*Tragelaphus strepsiceros*), nyala (*Tragelaphus angasii*), bushbuck (*Tragelaphus strepsiceros*), impala (*Aepyceros melampus*) and grey duiker (*Silvicapra grimmia*).
Here we did two studies: (1) a study in which we compared tree community composition and tree trait values between mound and control plots (hereafter: 'mound study') and (2) a study on the feeding preferences of different browser species (hereafter: 'browser preferences study').

### 6.3.2 Mound study: plot selection

During November and December 2009, sixteen sites were selected stratified at random at different locations within HiP. Sites were within 500 m of a road (for safety reasons), at least 500 m away from larger rivers, and away from drainages and at least 600 m apart from each other, with the mean nearest neighbour distance being 3.4 km (S6.1). At each *a priori* selected site the nearest *Macrotermes* spp. mound was selected for this study. A mature termite mound was recognized by its central dome-shaped structure surrounded by a cone shaped erosion skirt. We defined a mound plot as the 10 x 10 m area around the centre of the mound. Although most mounds were smaller than 10 x 10 m, it is likely that within this area the vegetation was highly altered by *Macrotermes* activities, since the influence of *Macrotermes* termites on plant communities goes well beyond their own mound, e.g., through subterranean foraging tunnels (Levick et al. 2010). By surveying 16 relatively small sites that were at relatively large distances from each other, we made sure that any statistical differences found between mound and control plots in our study represented patterns that were generalizable for our whole study area, rather than differences resulting from pseudoreplication.
The 16 control plots, also measuring 10 x 10 m, were selected by taking a random distance between 20 and 80 meters from the centre of the termite mound in a random compass bearing (1-360°). Obvious landscape features, such as wallows, were avoided. In case that another *Macrotermes* mound would be closer to the control plot, a new random distance and direction were taken.

### 6.3.3 Mound study: tree monitoring

Surface coverage of grass, forbs and bare soil and average height of the vegetation were estimated for both mound and control plots. Then, all trees above 0.5 m in height in the plots were identified to species level using Pooley (1997), local experts and the herbarium collection of the park. Canopy height of individual trees was estimated using 2 classes: above and below 2 meters in height. This 2 meter cut-off has been shown to reflect a cut-off between high and low mortality by fires (Higgins et al. 2007) and furthermore, it has been shown that except for giraffes and elephants, browsers hardly feed from vegetation above 2 m (Du Toit 1990). Stem diameter at stump level of trees was estimated using 7 classes: (1) 0 – 1 cm; (2); 1-3 cm; (3) 3-10 cm; (4); 10-20 cm; (5) 20-30 cm; (6); 30-40 cm; and (7); 40-50 cm. Tree diameter levels were used to calculate the total surface area (TSA) of each species in a plot: $TSA = \sum_{i=1}^{n} \pi r_i^2$ in which n is the number of individuals and r is the average radius from the diameter class the i-th individual belongs to (e.g. 0.25 cm for class 1). TSA was used as a proxy for the biomass of the given species in a plot.

### 6.3.4 Mound study: trait selection

At each site, 22 traits were measured for all tree species, representing functional adaptations to drought, fire, herbivory resistance and attractiveness, light competition and nutrient limitations. The traits examined were specific leaf area (SLA), leaf area (LA), leaf fractal dimension (FD), thorn length (TL) and density (TD), branch angle (BA), leaf trichome density (TrD), wood density (WD) and leaf polyphenol, carbon (C), nitrogen (N), phosphorous (P), sulphur (S), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), borium (B) and copper

(Cu) contents. For the functional significance of these traits, see Cooper & Owen-Smith (1986); Brown & Lawton (1991); Yates & Peckol (1993); Marschner (1995); Olff et al. (1999); Weiher et al. (1999); Hacke et al. (2001); Westoby et al. (2002) and Cornelissen et al. (2003).

### 6.3.5 Mound study: locations and sample size of trait measurements

Traits were measured for each species in each site where the species was found for SLA, LA, FD, TL, TD and BA (all 5 measurements per site), WD (3 measurements per site), SLA (5 – 30 leaves), LA (5 – 30 leaves), FD (5 – 30 leaves), leaf polyphenol, C and N content. TrD was measured for each species in 1 – 14 sites and leaf P, Na, Ca, Mg, P, S, Fe, Mn, Zn, B, and Cu concentrations were measured for each species in 1 – 7 sites. Ideally, trait values would have been measured across different sites for all species, but for the rarer species this was impossible. With our approach, we calculated species average trait values with as much replicates as possible. As a consequence, replication was thus higher for more common species. As many traits as possible were sampled from the same set of individuals in each plot.

Whenever possible, we measured these trait values on trees found off termite mounds, so that trait value differences between species reflected intrinsic species differences, not differences caused by the environment. Only when within a site, a species was *only* found on a termite mound, we measured traits of that particular species on individuals growing on the mound. By measuring almost all trait data on trees growing off mounds, our analysis focussed on characterising the mean trait value of each species across different sites, not on within-species variation, e.g., between plots or between termite mounds and surroundings. This is based on the assumption that intrinsic species differences are generally much larger than trait variation within species (Garnier et al. 2001). This assumption received some support by our data (S6.2).

### 6.3.6 Mound study: trait measurements

For LA, 5 – 30 leaves (depending on size and weight of leaves) from multiple individuals were taken in the field and photographed with a reference scale on the background. The software SigmaScanPro v 5.0 (Systat Software Inc., San Jose, California, USA) was used to measure LA and leaf FD (leaf perimeter / LA) on fresh leaves. Leaves were then dried at 50ºC for at least 48 h and weight was measured to 0.001 g precision. SLA was calculated by dividing the total LA by the dry weight of a leaf sample. TL was measured from at least 5 thorns and three individuals. TD was measured similarly (5 branches, 3 individuals per site) over 20 cm at the top of a branch. Both traits were measured at a height of 1.0 meter if possible, a height that most browser species can reach (Du Toit 1990). BA was measured for 5 individuals using a protractor. TrD was measured by counting the number of trichomes on a 50x50 mm leaf surface using an electron microscope. For WD, three branches of 20 cm long with a diameter between 2 and 13 mm were collected for each species per site and dried in the oven at 50ºC for at least 48 h. Then dry mass was measured and WD was calculated as: $WD = \frac{\pi r^2 L}{M}$ , with WD as wood density in $m^3kg^{-1}$, r the radius of the branch in m, L the length of the branch in m and M the dry weight of the branch in kg.

For chemical analyses, for each species in each site, bulk samples of leaf material were taken, dried at 50ºC for at least 48 h and ground with a ball mill. Polyphenol content, as a measure of generic chemical defence, was measured using the method described in Mole & Waterman (1987). Leaf C and N conctents were measured for each species in each site by taking five mg of finely ground leaf material (weighted to 1 µg precision) and measuring concentrations with a Carlo-Elba NA 1500 element analyser in duplo. The leaf contents of K, Na, Ca, Mg, P, S, Fe, Mn, Zn, B and Cu were measured at the laboratory of BLGG AgroXpertus in Wageningen via ICP-Atomic-Emission-Spectrometry conform NEN6966.

### 6.3.7 Mound study: calculating trait averages

For each species and each trait, values were usually measured in several sites. These different values were used to calculate an average species trait value (ASTV) (see S3 for values). When combining the

community composition data with ASTV data, we could calculate unweighted (based on presence-ansence data), abundance- and biomass weighted average trait values. Abundance-weighted average trait values ($\overline{at}$) were calculated with: $\overline{at} = \frac{\sum_{i=1}^{n} a_i t_i}{\sum_{i=1}^{n} a_i}$, in which n is the number of species found in the plot, $a_i$ the abundance of the i-th species and $t_i$ the ASTV of the i-th species. Biomass-weighted average trait values ($\overline{bt}$) were calculated with: $\overline{bt} = \frac{\sum_{i=1}^{n} b_i t_i}{\sum_{i=1}^{n} b_i}$, in which n is the number of species found in the plot, $b_i$ the biomass of the i-th species and $t_i$ the ASTV of the i-th species.

### 6.3.8 Browser preference study: plot selection

In June and July 2003 and 2004, tree communities in a total of 219 plots of 50x50 m, situated within all the different habitat types of HiP (Whately & Porter 1983), except for grassland, were monitored. Distance between adjacent plots ranged between 100 and 3410 m (S6.1). Sampling effort for different habitat types was proportional to habitat contribution of the total area of HiP (Fig. S6B).

### 6.3.9 Browser preference study: utilization by browsers of different tree species

In all plots, all trees between 0.5 - 2 m (a height that is mostly utilized by browsers) were recorded. Individual trees were identified using Pooley (1997), local experts and the herbarium collection of the park. Then, all entire individual trees were assessed for ungulate browser impact. Browser damage could be identified by characteristic browser cropping or stripping of branch ends (Estes 1991). For each tree species that was also monitored in the 'mound study' and that occurred in at least 50 'browser preference study' plots, the preference index (PI) for each browser was calculated: PI = ((BMO - BME) / BME), in which BMO is number of individual trees of the given tree species on which Browsing Marks were Observed, and BME is the number of individual trees of the given tree species on which Browsing Marks were Expected, i.e. the relative abundance of the tree species multiplied by the total number of tree individuals on which biting marks were observed. PI could be calculated for 26 tree species, which accounted for 72.3 % of the total number of individuals of the 'mound study' sites.

### 6.3.10 Data analysis

Non-metric Multi-Dimensional Scaling (NMDS) analyses, based on the 'Bray-Curtis' dissimilarity index (Bray &Curtis 1957), were performed to visualize differences in community composition of mound and control tree communities. Four NMDS dimensions were calculated, with 50 iterations. Furthermore, to investigate whether differences in species composition between mound and control sites were significant, a Permanova Analysis, using the sites as random blocks, was performed, based on the 'Bray-Curtis' dissimilarity index (Bray & Curtis 1957). To identify 'indicator species' for control and mound plots, Dufrene & Legendre's (1997) indicator value, using 100.000 randomizations, was calculated. To visualize relationships between traits, Principal Component Analysis (PCA) was performed with all scaled trait values as active variables and browser PI as a passive variable. PCAs rely on data-sets without missing values and therefore missing trait values were estimated using Multiple Imputation with Chained Equations (MICE). Simple Linear Regression Analyses were used to investigate the relationships between browser PI and functional traits of tree species. Paired Wilcoxon Signed-Rank Tests, using sites as blocks, were used to study differences in species richness and abundance patterns, vegetation coverage, vegetation height, (unweighted, abundance-weighted and biomass-weighted) trait averages (of all species, but also focusing on legumes or non-legumes only) and (unweighted, abundance-weighted and biomass-weighted) average browser PI values between mound and control plots. Unpaired Wilcoxon Signed-Rank Tests were used to compare the SATVs between species restricted to mound plots and species restricted to control plots. To reduce the chance of making type I errors, we performed false discovery rate analyses following the Simes (1986) procedure. All analyses were done using R-2.13.1.

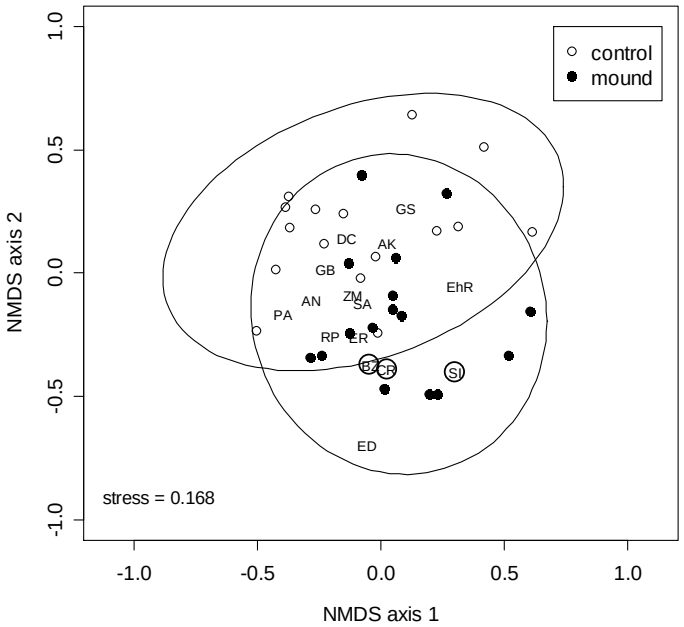


**Figure 6.1.** Biplot of the first two NMDS axes. Control plots are given in white, mound plots in black. The ellipses around the dots represent the 2-dimensional confidence interval (±2SD) of the average NMDS values of both mound and control plots. The fifteen most dominant species found in this study are shown in NMDS space with their abbreviations: AK = *Acacia karroo*, AN = *A. nilotica*, BZ = *Berchemia zeyheri*, CR = *Coddia rudis*, DC = *Dichrostachys cinerea*, ED = *Euclea divinorum*, ER = *E. racemosa*, EhR = *Ehretia rigida*, GB = *Gymnosporia buxofolia*, GS = *G. senegalensis*, PA = *Plectroniella armata*, RP = *Rhus pentheri*, SA = *Spirostachys africa*, SI = S*ideroxylon inerme*, ZM = *Zizyphus mucronata*. The species that were identified as indicator species for mound plots are encircled.

# 6.4 Results

## 6.4.1 Tree species composition, diversity and abundance and vegetation structure

In total, we found 67 tree species in our survey, of which 44 occurred in control plots and of which 59 occurred in mound plots. 23 Tree species were unique for termite mounds, while only 8 species were unique for control plots. 7 Indicator species (Dufrene & Legendre 1997) were identified for termite mound plots: *Gymnosporia senegalensis, G. nemorosa, Berchemia zeyheri, Sideroxylon inerme, Coddia rudis, Pappea capensis* and *Schotia brachypetala*, while there were no significant indicator species for control plots. Mound and control tree communities differed significantly in composition (PERMANOVA, $F = 2.050$, $P = 0.010$, Fig. 6.1). Tree communities of termite mounds were more species rich and had a higher Shannon-Wiener Diversity than the communities from the surrounding savannah, with on average 57% more species found in termite mound plots (Table 6.1, all $P < 0.05$). Overall abundance of trees did not differ between mound and control plots (Table 6.1, $P = 0.660$), although abundance of trees with a canopy height above 2 meters was 4.5 times higher on mound plots than on control plots (Table 6.1, $P = 0.023$). Termite mounds had a higher proportion of bare soil than control plots, an equal proportion of forb coverage as in control plots and a lower proportion of grass coverage than control plots (Table 6.1).

**Table 6.1.** Species richness, abundances, diversity and evenness: comparison of averages between control and mound sites. Standard errors are shown in parentheses. Abundance >2 m indicates the abundance of trees with a canopy height over 2 meter. Significance testing was done using a paired Shapiro-Wilkinson Test, with *V* as the test statistic and an associated *P*-value. P values below 0.05 that remained significant after false discovery rate testing are shown with an *, those that were not significant after false discovery rate testing are shown with an †.

| | control | mound | *V* | *P* |
|---|---|---|---|---|
| Species richness | 7.500 (0.816) | 11.81 (1.065) | 121.5 | 0.004* |
| Abundance | 127.8 (27.49) | 135.2 (14.94) | 77 | 0.623 |
| Abundance >2 m | 2.875 (1.258) | 12.94 (3.781) | 59 | 0.023* |
| Diversity | 1.208 (0.108) | 1.807 (0.131) | 125 | 0.002* |
| Evenness | 0.622 (0.038) | 0.740 (0.028) | 109 | 0.034† |
| % bare ground | 28.18 (5.96) | 45.09 (4.93) | 120.5 | 0.007* |
| % forb cover | 9.44 (2.25) | 9.59 (1.31) | 56 | 0.850 |
| % grass cover | 62.28 (5.36) | 45.31 (4.93) | 13 | 0.008* |
| grass height (cm) | 29.84 (4.14) | 22.81 (4.27) | 15 | 0.036† |
| forb height (cm) | 32.19 (5.16) | 26.56 (3.61) | 39 | 0.243 |

### 6.4.2 Relationships between functional traits

PCA analyses revealed that quantitative traits of tree species were relatively weakly correlated, so that even the strongest components explained a relatively low amount of total trait variation: the first and second PC respectively explained 19.0 and 14.1 % of total trait variance, and together 33.2 % (Table S6B). The first PC was positively correlated with leaf C content and negatively correlated with SLA, leaf N, P, K and S content, and therefore mostly related with variation in growth rate/resource use efficiency (Westoby et al . 2002). The second PC was mostly positively related with leaf FD, spine length and leaf N content, and negatively correlated with individual LA and leaf Na content. Therefore this PC mostly represented a 'typical legume -non legume axis' (Fig. 6.2, Table S6C).

### 6.4.3 Differences in trait values between mound and control trees

When focusing on species presence-absence-data (and thereby ignoring differences in species abundances between plots), tree communities on termite mounds had a lower average value of leaf FD, SD, and leaf polyphenol content, and higher values of leaf B content. Furthermore, mounds contained relatively less leguminous species (Table 6.2, all $P < 0.05$).

Tree communities on termite mounds contained species with a lower abundance-weighted average value of FD, SD, WD and leaf N, P and Zn concentration than species from control plots (Table 6.2, all $P < 0.05$). In contrast, LA and leaf Ca and Mg concentrations had a higher abundance-weighted average value on termite mounds (Table 6.2, all $P < 0.05$). Furthermore, mound plots contained a higher fraction of evergreen species and a lower fraction of leguminous species than control plots (Table 6.2, both $P < 0.05$).

Tree communities on termite mounds also contained species with a lower *biomass*-weighted average value of leaf FD and leaf N and Zn concentration than species from control plots (Table 6.2, all $P < 0.05$). In contrast, LA had a higher biomass-weighted average value on termite mounds (Table 6.2, $P < 0.05$). Furthermore, mound plots contained a higher biomass fraction of evergreen species and a lower biomass fraction of leguminous species than control plots (Table 6.2, both $P < 0.05$).

When studying differences in average trait values between mound and control communities, but only focusing on *either* legumes or non-legume species, most of the trait differences between mound and control species that were found when studying all species simultaneously, disappear. When comparing trait differences between species that were only found on either control termite or mound plots, hardly any significant differences were found.

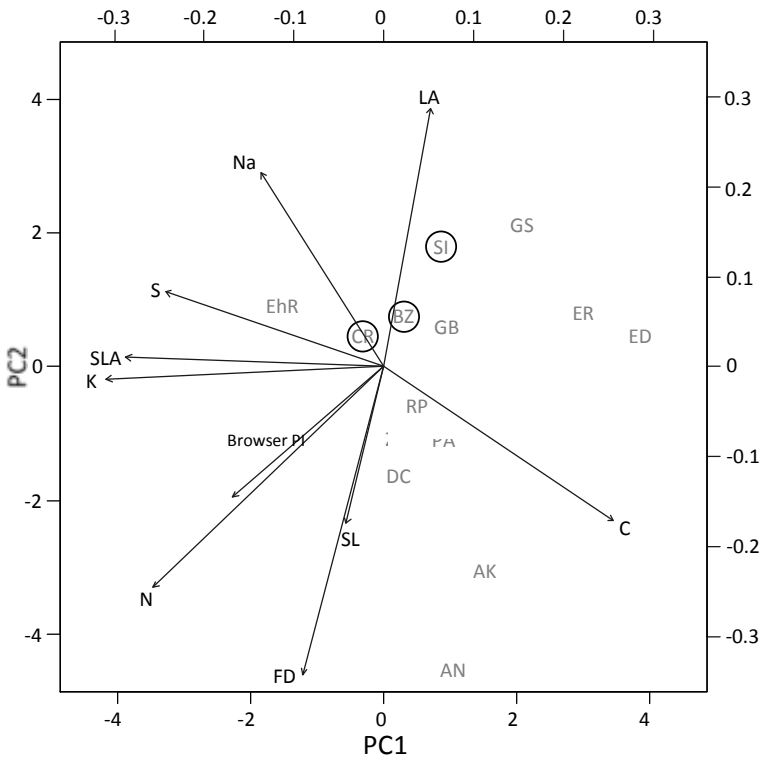


**Figure 6.2.** Biplot with the first two dimensions from the Principal Component Analysis (PCA). For the PCA, all measured, continuous trait variables (scaled) were used as active variables and browser PIs as passive variables. For each dimension, the five trait variables that correlated most strongly with it are plotted, as well as the browser PIs. Furthermore, the 15 tree species that were most abundant in the mound study are plotted. Abbreviations traits: LA = individual Leaf Area, C = leaf Carbon content, SL = Spine Length, FD = leaf Fractal Dimension, N = leaf Nitrogen content, K = leaf Potassium content, SLA = specific Leaf Area, S = leaf Sulphurous content and Na = leaf Sodium content. Abbreviations PIs: ele. = elephant PI, rhino = rhino PI, other = PI of 'other browsers' and

### 6.4.4 Relationships between browser PI, woody species trait values and habitat type

Although correlations were weak, browsers tended to prefer tree species with both low PC1 and PC2 scores (Fig. 6.2). Subsequent simple regression analyses revealed that browsers significantly preferred deciduous and leguminous trees ($T$ = 3.225, $P$ = 0.004, $R^2$ = 0.302 and $T$ = 2.081, $P$ = 0.048, $R^2$ = 0.153 respectively). Furthermore, browser PI correlated positively with tree species spine density ($T$ = 2.234, $P$ = 0.035, $R^2$ = 0.172), leaf P and N content ($T$ = 2.139, $P$ = 0.043, $R^2$ = 0.160 and $T$ = 2.769, $P$ = 0.011, $R^2$ = 0.242 respectively) and negatively with leaf Mg content ($T$ = -3.019, $P$ = 0.005, $R^2$ = 0.287). Consequently, tree communities on termite mounds had lower abundance- and biomass-weighted average ungulate browser PI values (Table 6.3, all $P$ < 0.05).

**Table 6.2.** Unweighted, abundance- and biomass weighted trait averages: comparison between control and mound sites. Significance testing was done using a paired Shapiro-Wilkinson Test, with *V* as the test statistic and an associated *P*-value. When the *P*-value was below 0.05 and the highest trait values were found in mound plots, the *P*-value is underlined. When the highest trait values were found in control plots, the *P*-value is bold. P values below 0.05 that remained significant after false discovery rate testing are shown with an *, those that were not significant after false discovery rate testing are shown with an †.

| Trait | unweighted trait average | | Abundance-weighted trait average | | Biomass-weighted trait average | |
|---|---|---|---|---|---|---|
| | *V* | *P* | *V* | *P* | *V* | *P* |
| specific leaf area ($cm^2\ g^{-1}$) | 79 | 0.597 | 72 | 0.860 | 46 | 0.274 |
| individual leaf area ($cm^2$) | 106 | 0.051 | 116 | 0.011* | 131 | < 0.001* |
| leaf fractal dimension ($cm^2\ cm^{-1}$) | 15 | **0.004*** | 11 | **0.002*** | 11 | **0.002*** |
| spine length (mm) | 28 | **0.039**† | 48 | 0.323 | 54 | 0.495 |
| spine density (no. spines) | 6 | **< 0.001*** | 20 | **0.011*** | 29 | **0.044**† |
| branch angle ( ) | 75 | 0.744 | 26 | **0.029**† | 69 | 0.980 |
| trichome density (trichomes $mm^{-2}$) | 74 | 0.782 | 72 | 0.860 | 48 | 0.323 |
| wood density ($kg\ L^{-1}$) | 33 | 0.074 | 9 | **0.001*** | 36 | 0.105 |
| polyphenol concentration ($g\ kg^{-1}$) | 19 | **0.009*** | 31 | 0.058 | 45 | 0.252 |
| C concentration (% dry weight) | 25 | **0.025**† | 27 | **0.034**† | 41 | 0.175 |
| N concentration (% dry weight) | 24 | **0.021**† | 15 | **0.004*** | 1 | **< 0.001*** |
| P concentration ($mmol\ kg^{-1}$) | 26 | **0.029**† | 12 | **0.002*** | 31 | 0.058 |
| S concentration ($mmol\ kg^{-1}$) | 86 | 0.375 | 61 | 0.744 | 61 | 0.744 |
| K concentration ($mmol\ kg^{-1}$) | 74 | 0.782 | 90 | 0.274 | 34 | 0.083 |
| Na concentration ($mmol\ kg^{-1}$) | 112 | 0.021† | 99 | 0.117 | 102 | 0.083 |
| Ca concentration ($mmol\ kg^{-1}$) | 97 | 0.144 | 136 | < 0.001* | 93 | 0.211 |
| Mg concentration ($\mu mol\ kg^{-1}$) | 110 | 0.029† | 121 | 0.004* | 104 | 0.065 |
| Fe concentration ($\mu mol\ kg^{-1}$) | 72 | 0.860 | 40 | 0.159 | 41 | 0.175 |
| Mn concentration ($\mu mol\ kg^{-1}$) | 69 | 0.980 | 101 | 0.093 | 65 | 0.900 |
| Zn concentration ($\mu mol\ kg^{-1}$) | 39 | 0.144 | 18 | **0.008*** | 9 | **0.001*** |
| B concentration ($\mu mol\ kg^{-1}$) | 124 | 0.002* | 103 | 0.074 | 76 | 0.706 |
| Cu concentration ($\mu mol\ kg^{-1}$) | 57 | 0.597 | 31 | 0.058 | 33 | 0.074 |
| Evergreen (no = 0; yes = 1) | 94.5 | 0.053 | 127 | 0.001* | 128 | < 0.001* |
| Leguminous (no = 0; yes = 1) | 15 | **0.004*** | 13 | **0.003*** | 8 | **< 0.001*** |
| Poisonous (no = 0; yes = 1) | 6 | 0.107 | 20 | 0.834 | 11 | 0.363 |

**Table 6.3**. Unweighted, abundance- and biomass weighted browser PI averages: comparison between control and mound sites. Standard errors are shown in parentheses. Significance testing was done using a paired Shapiro-Wilkinson Test, with *V* as the test statistic and an associated *P*-value. P values below 0.05 that remained significant after false discovery rate testing are shown with an *, those that were not significant after false discovery rate testing are shown with an †.

| Browser PI value | Weighted average control | mound | *V* | *P* |
|---|---|---|---|---|
| Unweighted average | 0.023 (0.025) | -0.038 (0.019) | 36 | 0.105 |
| Abundance-weighted average | -0.019 (0.026) | -0.092 (0.033) | 15 | 0.004* |
| Biomass-weighted average | 0.049 (0.038) | -0.103 (0.040) | 6 | < 0.001* |

# 6.5 Discussion

In this study, we used a trait-based approach to gain more insight in (1) the processes determining the characteristic community structure of tree communities on *Macrotermes* mounds in African savannahs and (2) why the trees from these communities are so attractive for browsers.

### 6.5.1 Differences between mound and savannah tree communities

We found that termite mounds supported unique tree communities which were more species rich and had higher species diversity than tree communities from the surrounding savannah, in line with results from Moe et al. (2009) and Traoré et al. (2008). Surprisingly, we did not find differences in overall tree densities between *Macrotermes* mound communities and communities from other places in the savannah. This is in contradiction with other studies (Loveridge & Moe 2004; Moe et al. 2009). However, when comparing the densities of trees with a canopy height above 2 m between termite mound and control plot communities, we found that termite mounds contained 4.5 times as many large trees. This suggests that the canopy coverage is also more closed at the height of 2 meters and that therefore, on mounds less light penetrates to lower layers than in the surrounding savannah, where light is highly available (Fig. 6.3) and unlikely to be limiting for tree growth. Reduced light penetration through mound vegetation could cause light competition and self-thinning to be much more important.

Not only did the tree species composition differ between termite mounds and the surrounding savannah, also the composition of functional traits was different. In general, termite mounds contained more evergreen, broadleaved species such as *Gymnosporia senegalensis, G. nemorosa, Berchemia zeyheri, Sideroxylon inerme, Coddia rudis, Pappea capensis* and *Schotia brachypetala*, while tree communities in the surrounding savanna were dominated by deciduous, fine leaved and spiny species from the leguminous Mimosaceae family, such as *Acacia spp*., *Dichrostachys cinerea* and *Ormocarpum trichocarpum*. This difference was also reflected by differences in average values of quantitative traits between mound and control communities: trees species on mounds had lower thorn densities and larger leaves with a lower leaf fractal dimension. In addition, tree species dominating on mounds had lower wood density and lower leaf nitrogen, phosphorous and zinc concentrations, but higher leaf calcium and magnesium concentrations.

There are several explanations of why leguminous species are relatively more common in surrounding savannah than on termite mounds. The main differences between Mimosaceae trees dominating most of the savannah and tree species dominating on mounds are that (1) most Mimosaceae species are nitrogen fixing; (2) spiny; (3) deciduous; (4) fine-leaved and (5) poor light competitors (Vitousek & Howarth 1991). *Macrotermes* mounds form relatively resource rich islands (Gosling et al. 2012), thereby reducing the need for trees to acquire extra nitrogen through nitrogen fixation or to protect tissue with spines from browsing (Coley et al.1985; Bryant et al. 1989; Vitousek & Howarth 1991). Also, although the evergreen leaves of tree species found on mounds are more costly to produce than deciduous leaves, on the long term, their long life expectancy is advantageous if (i) there is no strong

seasonal drought and (ii) if there are no imposed disturbances (such as fire) that destroy leaves (Givnish 2002). Indeed, termite mounds are known as areas with high water availability (Holt & Lepage 2000) and our data show that the surface of *Macrotermes* mounds consists of a high proportion of bare ground. This suggests a relatively low fuel load on termite mounds, resulting in lower impacts of fires on mounds. Also, our findings that tree species dominating on mounds had lower wood density and higher leaf area than species dominating the surrounding savannah, suggests that these mound tree species are less adapted to drought than tree species from the surrounding savannah. These traits reflect well known life-history trade-off strategies between high growth rates (for low wood density / large leaves) and high water use efficiency (for high wood density / small leaves) (Hacke et al. 2001; Westoby et al. 2002), implying that tree species with low wood densities and large leaves benefit from the higher nutrient and water availability on mounds. Finally, the finding that densities of tall trees are 4.5 times higher on termite mounds than in the surrounding savannah, suggests that these mounds might be among the rare places in savannahs where light competition plays an important role in the community assembly of trees. Nitrogen fixation is an expensive, carbon consuming process (Vitousek & Howarth 1991). Therefore, in the mound environment where competition for light is likely to be important, nitrogen fixation comes at a high cost, explaining the relatively low density of leguminous tree species.

It should be emphasized that these trait differences between tree species dominating on termite mounds and surrounding savannah mainly reflect trait differences between species from the leguminous Mimosaceae family, dominating on most of the savannah, and other tree species. When comparing trait differences between mound and control trees, but *only* taking *either* legume *or* non-legume species into account, or when only looking at presence-absence data, less differences in average trait values were found. Also, when comparing trait values of species unique for mound plots or control plots, hardly differences were found. This suggests that the trait differences we found between trees from mound and control plots, mainly reflect differences in the relative abundance of legume species on and off mounds. This is surprising, since species from the Mimosaceae family show high phylogenetic and functional relatedness. Therefore, one could expect them to have overlapping niches, making their co-existence problematic. We suggest that due to the harsh conditions (drought, fires) that make the savannah hostile for most other tree species, different species from the Mimosaceae family are not only able to co-occur in most of the savannah, but even in relatively high numbers. Termite mounds are among the rare sites in the savannah with also high abundances of other tree species that do not have the traits to cope with severe drought and fires.

**Figure 6.3.** Coverage of tall, dense vegetation is higher than in the surrounding on this dome-shaped *Macrotermes* mound. Note that the woody species on the mound hardly contain leaves below a height of ~1.5 meter, which is within reach of most browser species. Photo by Han Olff.

For tree species growing on relatively nutrient rich mound soils it seems counter-intuitive that the leaves contain lower nitrogen and phosphorous concentrations than the leaves from tree species dominating the surrounding savannah. However, there are 2 possible explanations for these results: (1) water limitation generally promotes plant nutrient concentration (Olff et al. 2002) and (2) abundances of leguminous, nitrogen fixing species on termite mounds are relatively low. This ability to fix nitrogen not only helps plant species to acquire nitrogen in nutrient poor environments, but also phosphorous (Houlton et al. 2008).

### 6.5.2 Tree traits and habitat and food preferences of browsers

For browsers, these differences in leaf N and P contents between tree species dominating on control plots and mound plots are crucial. Browsers preferred to feed on deciduous and leguminous tree species with high N and P contents. These results suggest that browsers should preferably feed on tree species that do *not* dominate termite mounds. This was indeed the case: browser PI values were higher for tree species dominating typical savannah vegetation that for tree species dominating *Macrotermes* mound vegetation. This is surprising, since other studies have demonstrated the function of termite mounds as browsing hotspots, at which a higher proportion of tree branches is consumed than in the surrounding vegetation (Holdo & McDowell 2004; Loveridge & Moe 2004), browser densities are higher (Mobæk et al. 2005; Brody et al. 2010) and in which the vegetation is more affected by browsing (Levick et al. 2010). Out of these studies, Holdo & McDowell (2004) were the only ones who also analyzed some nutrient contents in the foliage. They found that leaves from termite mound trees contained more P, K, Mg and Ca and suggested that this might explain their finding that elephants prefer to feed on trees growing on termite mounds. Our data also show higher Ca and Mg contents on tree species dominating termite mounds, but a *negative* relationship between Mg content and browser preference index, and *lower* P contents on tree species dominating termite mounds. Given these results, we conclude that differences in the tree community composition between termite mounds and the surrounding savanna do *not* explain the high browsing rates of mound vegetation found in other studies. The question thus remains why termite mounds can function as browser hotspots.

One answer could simply be: they actually not always do so. To the best of our knowledge, all published studies that did compare browser intensities (Mobæk et al. 2005; Brody et al. 2010), browser densities (Holdo & McDowell 2004; Loveridge & Moe 2004) and vegetation effects by browsers (Levick et al. 2010) on and off mounds, suggest that browsers prefer to feed on *Macrotermes* mounds. However, the mounds in our study site, which were dominated by non-preferred species, could be an exceptional case in which browsers do not prefer, and possibly even deter, mound vegetation. The soils in HiP are relatively nutrient rich when compared to soils in other savannah reserves, which might partially explain why the vegetation on mounds in our system does not have elevated nutrient levels when compared with the surrounding. Another possible, more likely, explanation could be that *within* species, tree individuals growing on termite mounds are more attractive to browsers than trees growing in the surrounding savannah. Soils on *Macrotermes* mounds in general (Holt & Lepage 2000), and also in our specific system (Gosling et al. 2012), are more rich in many nutrients than in the surrounding savannah. This could lead to similar nutritional differences between individuals from the same species growing on and off termite mounds, as shown by Gosling et al. (2012). Other possibilities are that during the warmest periods of the day, browsers prefer to forage in areas with dense vegetation that provide shelter, even if these same areas provide relatively low quality food, or that browsers select areas with high quantities of food to forage.

## 6.6 Conclusions

In summary, tree species growing on termite mounds seem to be less adapted to nutrient deficiency, drought and fire and more to light competition than tree species dominating the surrounding savannah. This is in line with other studies describing termite mound soils as nutrient-rich areas with high water availability and low frequencies of fires. Within an environment of stress, *Macrotermes* termites thus 'engineer' benign islands of fast tree growth, thereby promoting local scale heterogeneity in savannahs and contributing to their biodiversity. Despite the nutrient rich soils of mounds, the foliage of tree species growing there is relatively nutrient poor and not preferred by ungulate browsers. Since *Macrotermes* mounds are known as 'browsing hotspots', this is a surprising finding, and more research on intraspecific trait differences between trees growing on and off mounds could possibly shed more light on this.

## 6.7 Acknowledgements

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# Chapter 7: Responses of savannah lawn and bunch grasses to water limitation

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## 7.1 Abstract

The grass layer of African savannahs consists of two main vegetation types: grazing lawns, dominated by short and mostly clonally reproducing grasses, and bunch grasslands, dominated by tall and sexually reproducing bunch grasses. This patchy distribution of vegetation types is mostly created by large mammalian herbivores, which selectively feed on the more nutritious lawn grass species. Besides grazing, herbivores trample the soil, thereby causing soil compaction, with possible consequences for water infiltration. This raises two important questions: is water more limiting in grazing lawns than in bunch grasslands and if so, are lawn grasses more drought tolerant than bunch grasses?

To study these questions, we compared drought conditions in both lawn and bunch grasslands in a South African savannah. Additionally, in a climate room, we compared the performance of three lawn and three bunch grass species under a control and a drought treatment. Thirdly, we investigated whether there were differences in traits related to drought tolerance between lawn and bunch grasses.

Our results show that despite large differences in water availability in the field, lawn and bunch grasses did not differ from each other in their growth response to drought. Drought reduced growth of both growth forms equally. However, we found strong intrinsic trait differences between the two growth forms, with lawn grasses having higher Specific Root Length and Relative Growth Rate and bunch grasses having a higher root:shoot ratio. These results suggest that lawn and bunch grasses have different strategies to cope with drought.

## 7.2 Introduction

African savannahs are famous for their high abundances of large grazers (McNaughton 1985). These grazers significantly alter their surrounding by creating areas of short vegetation (McNaughton 1984; Hagenah et al. 2009), locally increasing nutrient inputs with their dung (Archibald et al. 2005) and by trampling the soil (Belsky et al. 1986; Dunne et al. 2011). These habitat modifications are, however, not homogeneously distributed over the landscape, but patchy, thereby contributing to the habitat heterogeneity in savannahs (Scholes 1993) and their associated high biodiversity (Mittermeier et al. 1998). One attribute of this heterogeneity is the patchy distribution of two different grassy vegetation types: lawn grasslands and bunch grasslands (Archibald et al. 2005; Cromsigt & Olff 2008; Stock et al. 2010).

Lawn grasslands are dominated by short, mostly clonally reproducing grass species (McNaughton 1983). Lawn grass species are usually highly nutritious (Bonnet et al. 2010) and therefore preferred by many vertebrate grazers (Kleynhans et al. 2011), which spend more time foraging on the lawn grasslands than on bunch grasslands (Cromsigt & Olff 2008). Bunch grasslands, on the other hand, are dominated by mostly sexually reproducing bunch grass species, which are usually less nutritious (Bonnet et al. 2010) and less preferred by several grazers, especially in the wet season (Kleynhans et al. 2011).

Several studies suggest that lawn grasses and large grazers have coevolved through mutual benefits (McNaughton 1984; Bouchenak-Kelladi et al. 2009). While exerting a negative short-time effect during defoliation, herbivores are suggested to positively affect lawn grass performance, by inducing (over)compensatory growth and promoting nutrient cycling (McNaughton 1984,1992; Anderson

2006). As a result, lawn grasses are expected to have a competitive advantage over bunch grasses in grazed systems (McNaughton 1984).
Large grazers might also impose indirect effects on savannah grasses, through altering soil conditions. African savannahs are highly trampled by large herbivores (Cumming & Cumming 2003), and like defoliation patterns, associated trampling patterns are patchy (Belsky 1986). The soil compaction resulting from trampling usually reduces water infiltration (Belsky 1986; Dunne et al. 2011). On top of that, highly grazed areas in savannahs often contain patches of bare soil with high microclimates (Metzger et al. 2005), so that water evaporates mostly through the soil instead of through evapotranspiration (Braud 1998). Questions arising from these studies are whether herbivores reduce water availability in grazing lawns through the creation of patches of bare, compacted soil, and whether this has led to higher drought tolerance in lawn grasses. If lawn grasses have indeed coevolved with herbivores, one would expect them to have adaptations to cope with drought.
To test these ideas, we first performed a field study in an African savannah to compare soil moisture, evaporation and water infiltration levels between bunch and lawn grasslands. Then we performed a climate room experiment in which we compared the growth response of three lawn and three bunch grass species to a one-month drought period. Additionally, for all grasses, we measured several functional traits related to water uptake and storage. As such, we investigated the following four questions: (1) is water more limited in grazing lawns than in bunch grasslands; (2) are savannah lawn grass species more tolerant to drought than savannah bunch grass species; (3) are lawn grasses better able to recover from a drought period; and (4) do lawn grass species differ in their traits from bunch grass species in such a way that they should be better able to capture / conserve water?

# 7.3 Methods and Materials

## 7.3.1 Field study: study site and plot selection

Our field study was performed in Hluhluwe-iMfolozi Park (HiP), South-Africa (28°00'-28°26'S, 31°41'-32°09'E). HiP is a ~90.000 ha park, with altitude ranging from 50 to 500m and annual amount of rainfall from ~450 till ~750mm per year. Rainfall mostly falls in the wet season (October-April), while rainfall is very limited from May till August. Vegetation types vary from grasslands, savannahs, broad leaved thickets, to forests (Whateley & Porter 1983). The grassland vegetation types can be categorized into 'bunch grasslands', dominated by tall grass species such as *Eragrostis curvula* and *Sporobolus pyramidalis*, and 'grazing lawns', dominated by short, clonally reproducing grass species such as *Digitaria longiflora* and *Sporobolus nitens* (Archibald et al. 2005; Stock et al. 2010).
In this park, 22 sites were visited. Sites were chosen along a rainfall gradient and with different parent materials (dolerite, sandstone or shale), based on geology maps of the park. Furthermore, sites were away from large rivers, outside the southern 'wilderness' area of HiP and at least at 100m away from each other. Sites were visited in two periods: July 2010 (dry season), and November 2010 – January 2011 (wet season). During these periods, evaporation, soil moisture content and water infiltration were measured in both grassland types.

## 7.3.2 Field study: measurements

During both the dry and wet season, the maximum rate of water infiltration in both grazing lawns and bunch grasslands was measured at each site, with a single replicate for each vegetation type. Measurements were done using a double-ring infiltrometer (Bower 1986). The inner ring (Ø10cm) was placed in the middle of the outer ring (Ø30cm) and hammered to a depth of about 30mm. Then first the outer ring was filled with water, followed by the filling of the inner ring. At the moment the inner ring was filled, timing was started. Using a measuring index at the side of the inner ring, the drop in water level in the inner compartment was recorded over time. Water level in the outer ring was maintained at the same level as the inner ring. Maximum time of the measurement was 10min or when all water had infiltrated into the soil.
In July, upper soil layers in both lawn and bunch grasslands were very dry. During this period, we measured potential soil evapotranspiration at both grassland types in 10 out of the 22 sites. 3 Replicates (~10m away from each other) of gypsum blocks were buried 10cm below the soil surface

in both lawn and bunch grass vegetation. 200 mL of water was added to soak both the gypsum block and the surrounding soil. After burying the gypsum blocks and also during the five following mornings, the electric resistance (in kΩ) of the gypsum block was measured with an Eijkelkamp 14.22 Soil Moisture Meter ®. Electric resistance was a proxy for moisture content, and measurements were compared to the initial measurement to calculate the relative reduction in moisture content. Measurements of the 3 replicates within sites were averaged in order to avoid pseudoreplication. No rainfall was recorded during the measurement days.

Also, soil moisture content was measured during the dry season. For this, at each site and each vegetation type, three replicate blocks of soil (~5x5x5 cm) were dug out, put in a closed plastic bag, taken to the HiP Research Centre, and fresh weight ($FW_{soil}$) was measured. After that, blocks were put in an oven at 105°C for 48h, after which dry weight ($DW_{soil}$) was measured. Soil moisture content was then calculated as: $\frac{FW_{soil} - DW_{soil}}{DW_{soil}}$. Again, measurements of the 3 different replicates within sites were averaged in order to avoid pseudoreplication.

In the wet season, measuring evaporation was impossible due to frequent rainfall events. Instead, we estimated soil moisture content using gypsum blocks. Soil moisture was measured at each site for both vegetation types, with a single replicate for each. Gypsum blocks were fully soaked and dug to a depth of 10cm in the soil. We did the first electric resistance (in kΩ) measurements with the Eijkelkamp 14.22 Soil Moisture Meter® 3 days after burrowing the gypsum blocks, assuming that by that time, moisture levels in the gypsum blocks had synchronized with moisture levels in the soil. Electric resistance was used as a proxy for soil moisture content, and was measured every week, at all sites on the same day, during our study period.

Additionally, we also measured daytime soil temperature in the wet season at all sites in both vegetation types, with three replicates for each vegetation type. This was done using iButtons. These were attached to a small metal plate marked with a plastic colour tie and dug to a depth of 10cm in the soil. Measurements of the 3 different replicates within sites were averaged in order to avoid pseudoreplication.

### 7.3.3 Climate room studies: grass collection

Three lawn and three bunch grass species were collected in January 2009 from several locations within HiP. Complete plants tillers were dug out and brought alive to the University of Groningen (Netherlands). The grass species collected for this experiment were: *Cynodon dactylon*, *Dactyloctenium australis*, *Digitaria longiflora* (lawn grasses) and *Eragrostis curvula*, *Setaria sphacelata* and *Sporobolus pyramidalis* (bunch grasses). We chose these species because they are abundant throughout the park (Hagenah et al. 2009; Stock et al. 2010) and good representatives of lawn and bunch grass growth forms. In the greenhouse, conspecifics from different locations (different genotypes) were grown in different pots. We suppressed sexual reproduction of these plants, and promoted vegetative growth and the production of new, genetically identical ramets.

### 7.3.4 Drought experiment: experimental set-up

We performed the drought experiment between November 2011 and January 2012. Of the species *D. australis*, *D. longiflora*, *E. curvula* and *S. sphacelata*, we selected three unique genotypes for our experiment, while four genotypes were used for *C. dactylon* and *S. pyramidalis*. From each genotype, we isolated 6 ramets, of which three were planted together in a 'control treatment' pot and the remaining in a 'drought treatment' pot. In total, we thus had 4 species (*D. australis*, *D. longiflora*, *E. curvula* and *S. sphacelata*) x 3 genotypes x 2 treatments + 2 species (*C. dactylon* and *S. pyramidalis*) x 4 genotypes x 2 treatments = 40 pots that were used in the experiment, each containing 3 individual ramets.

Before potting individual vegetative ramets, they were clipped to make them more or less equally sized (belowground length: 7cm, aboveground length: 10cm) and their fresh weight ($FW_{start}$) was measured. Also, for each species, 5 extra individuals were clipped, fresh weight was measured and they were dried at 70°C for 48h, after which dry weight was measured. These measurements were

used to make a calibration line between fresh and dry weight for each species, to estimate initial dry weight ($DW_{start}$) for plants used in the experiment.
Ramets were planted in PVC pots with a height of 30cm and a diameter of 10.5cm, filled with a soil mix containing 50% sand, 40% peat and 10% clay. At the bottom, a nylon mesh was put with a perforated PVC cap underneath it, allowing excess water to escape, while locking soil in. To limit soil evaporation, the soil of the PVC tubes was covered with aluminum foil, with small holes around the plants. The pots were placed in a climate room. Also, five extra pots ('soil only pots') were placed in the climate room, with the same soil and moisture content, and with aluminum foil containing some small holes, but without plants, to measure soil evaporation. The climate room had a 12/12 hours light/dark cycle. During the light period, the room was illuminated by Philips GreenPower DR/B LED lights®, with wave length peaks at 470 and 670nm, causing a light intensity of approximately 600 µmol $m^2s^{-1}$ at pot height. Temperatures during the light and dark period were 25 and 15˚C respectively.
During the first 3 weeks of the experiment ('initial growth period'), pots were watered 3 times per week in order to maintain a soil moisture content of 30% (i.e. 30mL water:100g soil). Once per week, pots were watered with a 1% Pokon® NPK solution to prevent malnutrition limiting plant performance. Starting in the fourth week, 'drought treatment pots' were not watered anymore for four weeks ('drought period'), while 'control pots' were still watered 3 times per week, without nutrient solution. These four weeks of drought corresponds to periods without rainfall that regularly occur in the dry season. After the drought period, all plants were watered again for 2 weeks ('recovery period'), after which the plants were harvested.

### 7.3.5 Drought experiment: measurements

Except during the drought period and except for the soil only pots, all other pots were watered 3 times per week. Just before watering, we measured the weight of the pot and compared that to the weight corresponding with 30% soil moisture content, in order to calculate the amount of total evaporation ($EV_{total}$). Then, pots were watered in order to obtain a 30% soil moisture content. For one week, we also measured the weight of the soil only pots in order to obtain the amount of soil evaporation ($EV_{soil}$). During the drought period, weight of drought pots was still measured and compared with the last measurement in order to calculate $EV_{total}$. Evaporation by the plants ($EV_{plants}$) was calculated as: $EV_{plant} = EV_{total} - EV_{soil}$. Once per week, we counted the number of leaves of each individual plant. We distinguished between alive (>50% green surface) and dead (<50% green surface) leaves. The total number of leaves was used to calculate relative leaf accumulation rate (RLAR) for both the drought and recovery period: $RLAR = \frac{\ln leaves_{end} - \ln leaves_{start}}{duration\ period\ (days)}$. Also, once per week, canopy height and maximum length were measured following Cornelissen et al. (2003).
At the end of the recovery period, all plants were harvested: they were removed from the pot and divided in 3 parts: aboveground parts, the upper 5cm of roots and deeper root parts. Root parts were gently rinsed with water to remove soil. Each plant part was weighted and dried in an oven at 70°C for 72h, after which dry weight of aboveground parts ($DW_{above}$), the upper 5cm of roots ($DW_{shallowroots}$) and of deeper roots ($DW_{deeproots}$) were measured. These were used to calculate total root dry weight ($DW_{roots}$) and total dry weight ($DW_{end}$), Absolute Growth ($AG = DW_{end} - DW_{start}$), Relative Growth Rate ($RGR = \frac{\ln DW_{end} - \ln DW_{start}}{duration\ experiment\ (days)}$) and Water Use Efficiency ($WUE = \frac{AG}{EV_{plant}}$), Root Weight Fraction ($RWF = \frac{DW_{root}}{DW_{end}}$) and Deep Root Weight Fraction ($DRWF = \frac{DW_{deeproots}}{DW_{root}}$).

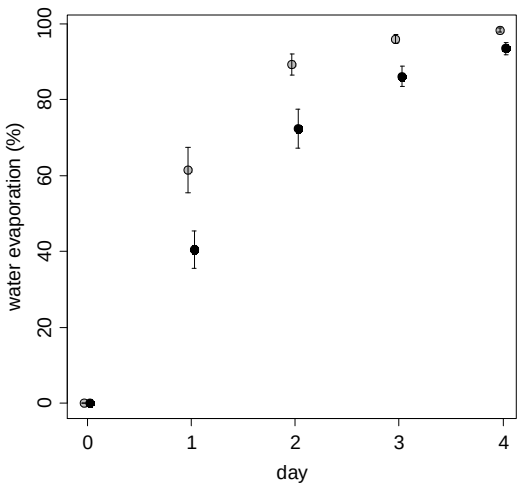
### 7.3.6 Specific Root Length (SRL) study: growth conditions and measurements

In order to measure SRL, we needed plants with perfectly clean roots. Therefore, for the same species as used in the drought experiment, we selected three unique genotypes to grow in a pot with 100% sandy soil. In total, we thus used 18 pots. Each pot contained 3 clones of the same genotype, clipped to 10 cm aboveground, 7cm belowground. The PVC pots had a height of 30cm and a diameter of 10.5cm. At the bottom, a nylon mesh was put with a perforated PVC cap underneath it, allowing

excess water to escape the PVC tube, but keeping sand locked in. To limit soil evaporation, the soil was covered with aluminum foil, with small holes around the plants. The pots were placed in a climate room with the same climatic conditions as in the drought experiment.
Pots were watered 3 times per week in order to maintain a soil moisture content of 22.5% (i.e. 22.5mL water:100g soil, approximately the water holding capacity of the sand). Once per week, pots were watered with a 1% Pokon® solution to prevent malnutrition limiting plant performance. After 6 weeks, plants were harvested and their roots were isolated from above ground parts and gently rinsed to get rid of remaining sand particles. Immediately after that, they were coloured in a 1% NeutralRed solution. Roots were then scanned using a Regent Positioning System (Epson® Expression® 10000 XL). Total length of roots was then estimated using WinRHIZO software. Roots were then dried in an oven at 70°C for 72h, after which their dry weight was measured. SRL was calculated by dividing the total length by dry weight of the roots. The SRL study took place in April and May 2012.

### 7.3.7 Statistical Analysis

We compared dry and wet season water infiltration and dry season evaporation between vegetation types using General Linear Mixed Models (GLMM), with full models containing vegetation type, time and their interaction effect as fixed factors and site as random effect. For wet season soil moisture content, we constructed a GLMM with the full model containing vegetation type as a fixed factor and site and time as random effects. For dry season soil moisture content and wet season soil temperature, we constructed a GLMM with the full model containing vegetation type as a fixed factor and site as a random effect. AG, RGR, WUE, $RLAR_{drought}$, $RLAR_{recovery}$, RRW, RDRW and SRL were compared between growth forms and treatments using GLMMs with full models containing growth form, treatment and their interaction effect as fixed factors and species and genotype (nested within species) as random effects. Soil moisture concentration and evaporation during the lab experiment were compared between growth forms and treatments using GLMMs, full models containing growth form, treatment, week and their interaction effects as fixed factors and species and genotype (nested within species) as random effects. We checked for assumptions of normality and log-transformed wet season water infiltration to meet this criterion. In some cases, the assumption of equal standard deviations between groups was violated: standard deviations differed between species for $RLAR_{drought}$ and $RLAR_{recovery}$, between growth forms for WUE and between species for AG. In these cases, we modeled equal variances following Pinheiro and Bates (2000). Using a Maximum Likelihood model selection procedure, we selected a final model with the lowest AIC value for each GLMM. From the data collected in our climate room studies, only individuals that gained at least one leaf in the initial growth period ('healthy individuals') and that did not grow in a drought pot with less than 2 healthy individuals were included in the statistical analyses, in order to prevent biases in the outcomes resulting from transplantation problems or relaxed drought treatment conditions. All statistics were performed using R (R Development Core Team 2011).



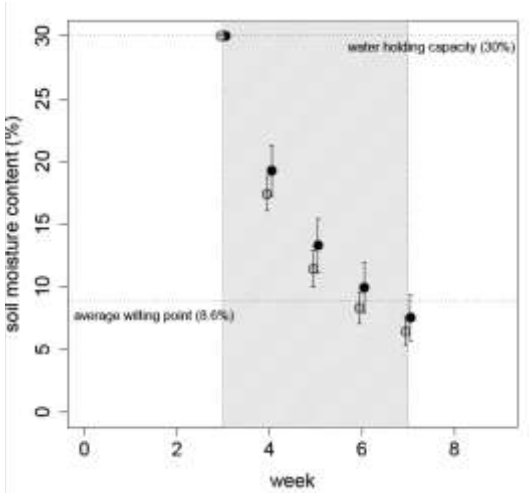




**Figure 7.1.** (left) Water evaporation in percentages of the initial amount of water. Measurements from bunch grasslands are depicted with circles, measurements from grazing lawns are depicted with triangles. **Figure 7.2.** (right) Soil moisture content in percentages. Data points depict measurements just after the moment that control pots were given water. Control treatment pots are depicted with circles, while drought treatment pots are depicted with triangles. The vertical, striped lines mark the drought period.

# 7.4 Results

## 7.4.1 Soil differences between grazing lawns and bunch grasslands

During the dry season, water infiltration was more than 50% lower in grazing lawns than in bunch grasslands (Table 7.1). Also, water evaporated significantly more rapidly in grazing lawns than in bunch grasslands: approximately 90% of the water was evaporated in lawn grasslands after 2 days, while it took approximately 3.5 days before the same amount evaporated in bunch grasslands (Fig. 7.1; Table 7.1). Dry season soil moisture content was significantly higher in bunch grasslands than in lawn grasslands (Table 7.1). During the wet season, daytime soil temperatures were on average more than 7°C warmer in lawn grasslands than in bunch grasslands. Water infiltration was 46.0% lower in grazing lawns soils than in bunch grassland soils (Table 7.1). Soil moisture was significantly higher in bunch grasslands than in lawn grasslands (Table 7.1).

## 7.4.2 Effectiveness drought treatment on soil moisture content

From the 3$^{rd}$ till the 7$^{th}$ week of the experiment, drought treatment pots were not watered, while control treatment pots were watered 3 times per week in order to maintain soil moisture content of 30%. This led to a steady decrease in soil moisture content in the drought treatment in both bunch and lawn grass pots (Fig. 7.2; Table 7.2). Total pot evaporation between week 3 and 7 among drought plants did not correlate significantly with the estimated plant biomass in week 3 ($R^2$ = 0.053, $T$ = 1.625, $P$ = 0.111). The drought treatment also led to a decrease in daily evaporation between the 3$^{rd}$ and 7$^{th}$ week, while evaporation in control plots increased with time in the same period (Fig. 7.3; Table 7.2).

**Table 7.1**. Average values plus standard deviations for soil moisture levels and infiltration rates in lawn and bunch grasslands. On the right, the test statistics of final GLMMs are shown. The unit of evaporation index (%) indicates the percentage loss of electrical resistance when compared to initial measurements of fully soaked gypsum blocks.

| **Trait** | **Lawn** | **Bunch** | **Best model** significant fixed effects |
|---|---|---|---|
| evaporation index dry season (% ) | 68.978 ± 5.427 | 58.455 ± 5.137 | vegetation type: $F = 9.411$, $P = 0.003$; time: $F = 365.373$, $P < 0.001$ |
| soil moisture dry season (%) | 0.058 ± 0.006 | 0.064 ± 0.007 | vegetation type: $F = 5.517$, $P = 0.031$ |
| infiltration dry season (mm) | 1.592 ± 0.087 | 3.226 ± 0.144 | vegetation type: $F = 263.872$, $P < 0.001$; time: $F = 405.103$, $P < 0.001$;< 0.001; time: $F = 405.103$, $P < 0.001$; vegetation type x time: $F = 40.596$, $P < 0.001$ |
| soil moisture wet season (kΩ) | 51.224 ± 2.245 | 55.896 ± 2.232 | Vegetation type: $F = 6.7589$, $P = 0.010$ |
| infiltration wet season (log mm) | 0.528 ± 0.018 | 0.796 ± 0.018 | vegetation type: $F = 283.676$, $P < 0.001$; time: $F = 706.461$, $P <$ 0.001;81.907, $P < 0.001$; vegetation type x time: $F = 81.907$, $P < 0.001$ |
| soil temperature (°C) | 38.48± 1.48 | 31.21± 0.79 | vegetation type: $F = 50.614$, $P < 0.001$ |

**Table 7.2.** Average values plus standard deviations for traits measured for lawn and bunch grasses in the drought and control treatment. On the right, the test statistics of final GLMMs are shown.

| trait | lawn | | bunch | | Best modell | |
|---|---|---|---|---|---|---|
| | Ccontrol | drought | control | drought | Significant fixed effects | AIC |
| soil moisture week 3-7 (%) | 30.000 ± 0.000 | 14.727 ± 1.373 | 30.000 ± 0.000 | 15.864 ± 1.455 | week: $F = 346.580$, $P < 0.0001$ | 529.959 |
| daily evaporation week 3-7 (mL) | 11.950 ± 1.016 | 4.954 ± 0.562 | 9.872 ± 0.858 | 4.625 ± 0.498 | treatment: $F = 196.828$, $P < 0.0001$; week: $F = 383.552$, $P < 0.0001$; txw: $F = 107.129$, $P < 0.0001$ | 2008.176 |
| absolute growth (g) | 7.856 ± 1.036 | 3.661 ± 0.433 | 7.623 ± 0.981 | 4.289 ± 0.587 | treatment: $F = 24.292$, $P < 0.0001$ | 560.749 |
| relative growth rate (g $g^{-1}$ $day^{-1}$) | 0.044 ± 0.002 | 0.035 ± 0.002 | 0.035 ± 0.003 | 0.029 ± 0.003 | growth form: $F = 8.050$, $P = 0.0470$; treatment: $F = 13.687$, $P = 0.0004$ | -618.118 |
| water use efficiency (g $mL^{-1}$) | 0.011 ± 0.001 | 0.012 ± 0.001 | 0.012 ± 0.002 | 0.015 ± 0.002 | | -720.502 |
| RLAR drought (leaf $leaf^{-1}$ $day^{-1}$) | 0.065 ± 0.002 | 0.036 ± 0.002 | 0.039 ± 0.004 | 0.022 ± 0.002 | growth form: $F = 63.3687$, $P = 0.0013$; treatment: $F = 196.2553$, $P < 0.0001$; gxt: $F = 9.2253$, $P = 0.0032$ | -602.223 |
| RLAR recovery (leaf $leaf^{-1}$ $day^{-1}$) | 0.034 ± 0.002 | 0.051 ± 0.002 | 0.023 ± 0.004 | 0.034 ± 0.002 | growth form: $F = 26.2494$, $P = 0.0069$; treatment: $F = 61.7337$, $P < 0.0001$ | -585.761 |
| root weight fraction | 0.181 ± 0.023 | 0.177 ± 0.013 | 0.292 ± 0.017 | 0.294 ± 0.017 | growth form: $F = 11.3719$, $P = 0.0280$ | -204.856 |
| deep root weight fraction | 0.593 ± 0.036 | 0.577 ± 0.032 | 0.568 ± 0.038 | 0.546 ± 0.037 | | -53.795 |
| specific root length (m $g^{-1}$) | 50.92 ± 6.25 | | 26.16 ± 3.84 | | growth form: $F = 9.2098$, $P = 0.0386$ | 583.124 |

### 7.4.3 Effects of drought on plant performance

Drought had an equally negative effect on the absolute growth (AG) of lawn and bunch grasses, with AG being reduced by 48.9% for drought treatment grasses (AG control treatment: 7.742g; drought treatment: 3.956g; Table 7.2). Relative Growth Rate (RGR) was higher for lawn grasses than for bunch grasses (RGR lawn grasses: 0.0394 g $g^{-1}day^{-1}$; bunch grasses: 0.0319 g $g^{-1}day^{-1}$), but the effect of drought on RGR did not differ between lawn and bunch grasses, as both reduced with 19.5% (Fig. 7.4; RGR control treatment: 0.0395 g $g^{-1}day^{-1}$; drought treatment: 0.0318 g $g^{-1}day^{-1}$) (Table 7.2). WUE was not affected by drought and did not differ between growth forms.

During the drought period, Relative Leaf Accumulation Rate (RLAR) was on average 62.4% higher for lawn grasses than for bunch grasses (Fig. 7.5; RLAR lawn grasses: 0.0508 leaves $leaf^{-1}day^{-1}$; bunch grasses: 0.0313 leaves $leaf^{-1}day^{-1}$), but drought caused a stronger decrease in RLAR for lawn grasses than for bunch grasses (Fig. 7.5; Table 7.2). Also in the recovery period, RLAR was higher for lawn grasses than for bunch grasses (Fig. 7.5; RLAR lawn grasses: 0.0424 leaves $leaf^{-1}day^{-1}$; bunch grasses: 0.0285 leaves $leaf^{-1}day^{-1}$), but the growth forms did not differ in RLAR response during the recovery period after a drought treatment, both increased RLAR when they experienced drought in the period before (Fig. 7.5; Table 7.2).

### 7.4.4 Differences between root functional strategies

Drought did not affect the Relative Root Weight (RRW) of either growth form, although bunch and lawn grasses differed in RRW (Fig. 7.6), with bunch grasses on average having a 63.7% higher RRW than lawn grasses (RRW bunch grasses: 0.293; lawn grasses: 0.179; Table 7.2). Specific Root Length (SRL) was almost twice as high (94.7%) in lawn grasses as in bunch grasses (SRL bunch grasses: 2615.80 cm $g^{-1}$; lawn grasses: 5092.12; Table 7.2). Relative Deep Root Weight (RDRW) did not significantly differ between treatments or growth forms (Table 7.2). For a more complete overview of the values of all traits measured during this study, see Table S7.1A in S7.1).

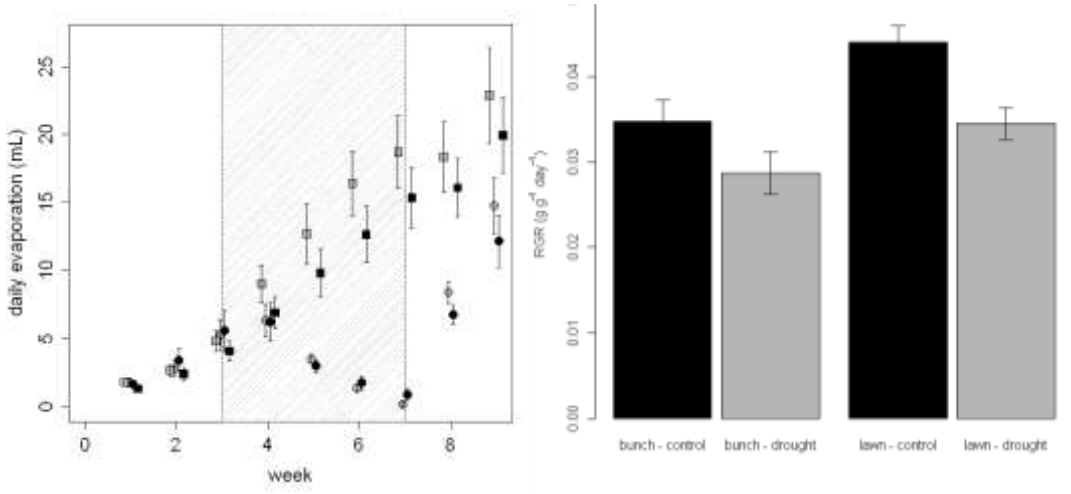



**Figure 7.3**. (left) Daily evaporation in mL. Data points depict weekly averages. Control treatment pots are depicted with circles, while drought treatment pots are depicted with triangles. The vertical, striped lines mark the drought period. **Figure 7.4**. (right) Mean Relative Growth Rate ± standard error of lawn and bunch grasses in the control and drought treatment.

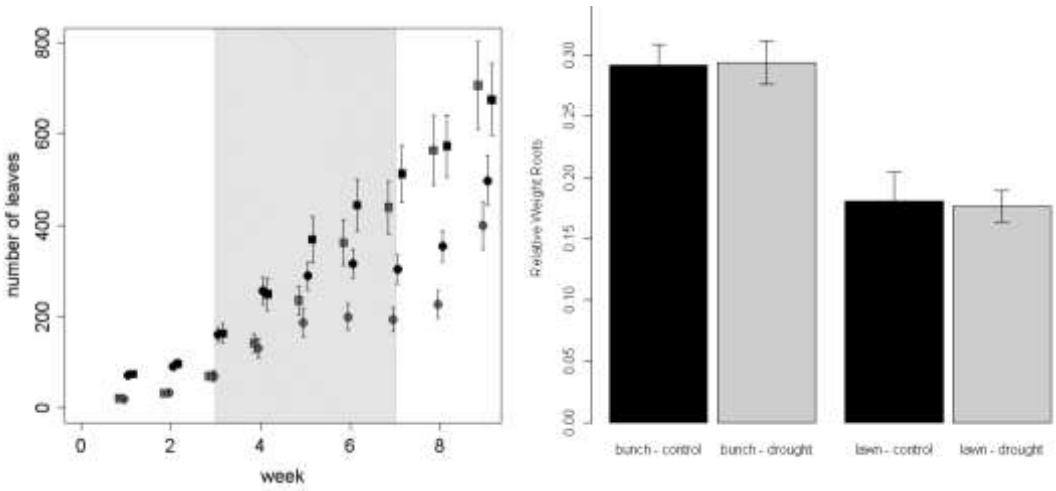



**Figure 7.5.** (left) Number of leaves per plant of bunch (top) and lawn (bottom) grasses through time. The vertical, striped lines mark the drought period. **Figure 7.6** (right). Mean Relative Weight Roots ± standard error of lawn and bunch grasses in the control and drought treatment.

## 7.5 Discussion

The first goal of this study was to investigate whether grazing lawn grasses are more exposed to drought than bunch grasses. We expected that the presence of grazers and their effects on soil compaction, bare patch formation (Belsky 1986; Braud 1998; Dunne et al. 2011) and elevated soil temperatures would lead to increased levels of water evaporation and decreased levels of water infiltration and moisture content in grazing lawn soils. Our field study confirmed these expectations: water infiltration was much lower in grazing lawn soils than in bunch grass soils in both seasons. Furthermore, dry season soil water evaporation was higher in grazing lawns, probably because of the high daytime temperatures observed there. These results suggest that after a rain event, grazing lawn soils receive less water than bunch grass soils. Furthermore, at the beginning of the dry season, grazing soils should dry out more quickly than bunch grassland soils. Significantly higher soil moisture values were indeed observed in bunch grasslands than in lawn grasslands, although these differences were relatively moderate. Possibly, these differences are larger during the transitions between seasons.

Because in the field lawn grasses are more exposed to drought than bunch grasses, we expected that lawn grasses would be more drought resistant. However, although our experiment showed that a drought period leads to a rapid depletion of soil water, the rate was more or less equal for lawn and bunch grasses. Both growth forms failed to become more efficient with their water use, and therefore drought led to a sharp decrease in the both absolute and relative growth rate (RGR) in growth forms. Thus surprisingly, lawn grasses were not more tolerant to drought than bunch grasses in our experiment, given the duration of the drought we imposed.

Our third goal was to investigate whether lawn and bunch grasses differed in their direct response to drought itself or in their response to a recovery period after drought. Other studies have shown that plants can differ remarkably in drought and post-drought performance (Chai et al. 2010). Our results showed that both during the drought and the recovery period, lawn grasses had a much higher relative leaf accumulation rate (RLAR) than bunch grasses. Furthermore, both growth forms had a increased RLAR in the recovery period when they had been exposed to drought before, which partially

compensated for the reduced growth during drought (Fig. 7.4). One difference between lawn and bunch grasses is that the decrease in RLAR when experiencing drought is stronger for lawn grasses than for bunch grasses. However, this result seems to arise mostly from the large absolute difference between lawn and bunch grasses in RLAR. When comparing their relative decrease in RLAR when experiencing drought, these are 44% and 43 % for lawn and bunch grasses respectively and thus hardly different. It therefore seems that in our experiment, lawn and bunch grasses did neither differ from each other in their growth response to the drought period nor in their growth response in a post-drought recovery period. Instead it might be the inherently higher growth potential of the lawn grasses that are most important for growing in grazed areas.

The last goal in this study was to compare functional traits related to water uptake and storage between growth forms. Irrespective of whether plants differ in their response to drought, they can have very different traits to reach similar performance (Marks & Lechowicz 2006). Here, we compared three key root traits between lawn and bunch grasses: Root Weight Fraction (RWF), Deep Root Weight Fraction (DRWF) and Specific Root Length (SRL). RWF and DRWF are important determinants of water uptake capacity (Schulze et al. 1996; Asseng et al. 1998; Huang 1999) and SRL is functionally positively related to root growth, RGR (Reich et al. 1998) and water uptake (Huang & Eissenstat 2000). Our results showed that lawn and bunch grasses allocated similarly to deeper roots. However, the total allocation of biomass to roots was higher for bunch grasses, while lawn grasses had much thinner roots. This may reflect different strategies to cope with drought stress: less but more efficient roots in lawn grasses versus more but less efficient roots in bunch grasses. Therefore we conclude that in our experiment, bunch and lawn grasses had different strategies to cope with water stress, but these different strategies converged to similar tolerance to short-term drought stress.

It is surprising that despite the large differences found in water infiltration and evaporation between grazing lawns and bunch grasslands, the dominant species of these habitats showed similar drought tolerance. Several explanations are possible for this: i) these habitat differences have indeed not led to differential adaptation to drought and the growth forms cope in different ways with this, ii) growth forms only differ in drought tolerance when simultaneously being exposed to herbivory, and iii) differences in drought tolerance may only become important after longer drought periods.

Although growth forms did not show differences in drought tolerance in our experiment, they might differ in their capacity to recolonize patches of bare soil. In field conditions, in the drier lawn grasslands, more plants are expected to die. If so, lawn grasses need to recolonize lost patches in order not to be replaced by bunch grass vegetation. Lawn grasses had higher RGR than bunch grasses, and this in combination with their horizontal growth and clonal reproduction (McNaughton 1992), may facilitate lawn grasses to recolonize bare patches of soil. This could happen early in the wet season, before bunch grasses have produced seeds. Thus perhaps lawn grasses are not adapted to drought *per se*, but to recolonization after drought.

Another possibility is that lawn grasses do differ from bunch grasses in their tolerance to drought, but only when simultaneously exposed to herbivory. The lawn grasses in our experiment had a relatively high RGR and SRL. While a high RGR is important for quick regrowth after defoliation, a high SRL helps to reach high nutrient concentrations at the same time (McNaughton 1992; Anderson et al. 2006). These high nutrient levels attract the grazers that maintain the preferred growing conditions of lawn grasses (McNaughton 1984; Cromsigt & Olff 2008; Kleynhans et al. 2011). Under field conditions, where lawn grasses are defoliated often, lawn grasses may not have such a low root:shoot ratio as measured in our experiment. This could lead to lower evaporation and therefore higher drought tolerance. This implicates that while lawn grasses are not more tolerant to drought than bunch grasses *per se*, they may be so when drought coincides with defoliation. Coughenour et al. studied interaction effects between defoliation and drought on plant growth in climate rooms and found that for one species, plants suffered less from drought when clipped (Coughenour et al. 1990), while for another species, *Themeda trianda*, a common bunch grass in African savannahs, plants did not suffer less from drought when being clipped (Coughenour et al. 1985). Thus, when simultaneously experiencing grazing and drought, lawn grasses might perform better than bunch grasses.

A third possibility is that the drought period in our experiment was too short. We exposed grasses to a drought period of one month. In the field, periods without rainfall of longer than one month do occur (KZN Wildlife, unpublished data). Lawn grasses might be more capable than bunch grasses to survive extreme periods of drought, by reallocating important nutrients and minerals from their above-ground tissue to below-ground parts, thereby 'sacrificing' above-ground tissue and 'hibernating' underground. In that case, a short term effect of drought on lawn grass performance would be reduced growth and increased die-off of above-ground plant parts, but longer term effects would be limited.
Summarizing, our results showed that despite large differences in water availability between grazing lawns and bunch grasslands, in our experiment, the dominant species of these vegetation types did neither differ in drought tolerance nor in post-drought recovery. Lawn and bunch grasses do, however, differ in traits related to drought tolerance and growth rate. We suggest that these trait differences possibly reflect differences between savannah lawn and bunch grasses in their capacity to recolonize areas after drought and in their tolerance to simultaneous exposure to drought and herbivory.

## 7.6 Acknowledgements

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## Section III: traits and community assembly: understanding the relative importance of different ecological processes

# Chapter 8: A new approach to quantifying the relative importance of different community assembly processes, illustrated for African savanna trees.

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## 8.1 Abstract

Ecological communities are shaped by a combination of stochastic and different niche-based (e.g. competitive) processes. The expression of these different processes in community assembly is often analyzed by comparing trait distributions of co-occurring species with null model predictions. 'Filtering processes', such as the exclusion of stress-intolerant species from particular habitats, increase similarity of traits of co-occurring species. Other processes 'limit similarity' of co-occurring species, for example, competition limits similarity of traits that are not related to invasion fitness. By comparing within-community trait similarity patterns of observed communities and those of 'randomized communities', one can thus detect the expression of filtering and limiting similarity processes.

However, such comparisons with null models do not inform us about the *relative contribution* of these different processes in shaping communities. Here, we develop a new approach using Approximate Bayesian Computation (ABC) that quantifies the relative importance of stochastic, filtering and limiting similarity processes for observed communities for which species composition and species traits have been measured. We applied this approach to tree community composition and trait data, collected at 20 locations over a rainfall and fire gradient in a highly heterogeneous South African savanna.

Our new approach suggests that stochastic processes are more important in shaping these communities than filtering and especially limiting similarity processes. In addition, we find limiting similarity processes to decrease in importance towards lower rainfall and higher fire frequencies, suggesting that under such conditions species resource partitioning is irrelevant. We find traditional null model comparisons to perform poorly in detecting any signs of limiting similarity. We conclude that our method holds promise for new future opportunities to quantify the relative importance of community assembly processes across different species groups, ecosystems and biomes.

## 8.2 Introduction

A central goal in community ecology is to understand the principles that govern the assembly of ecological communities. In order to do so, ecologists have traditionally focused on the importance of niche-based community assembly processes, such as the 'filtering out' of stress intolerant species under harsh abiotic conditions, and the loss of species with 'too similar' traits through competitive exclusion (Hutchinson 1959; Diamond 1975; Weiher & Keddy 1995a; McGill et al. 2006). Contrary to this view, others have drawn attention to the potential importance of stochastic processes for community assembly, such as random birth, death and dispersal events (Caswell 1976; Sale 1977; Hubbell 2001). Both views have led to some 'extreme' theoretical models, in which communities are solely shaped by either niche-based (e.g. Tilman 1982; Chase & Leibold 2003) or stochastic (e.g. Hubbell 2001) processes. In the past, this led to much discussion over which of these models reflects community assembly most accurately. Nowadays it is increasingly recognized that both stochastic and niche-based processes are important in community assembly (Vellend 2010; Weiher et al. 2011; Rosindell et al. 2011), although their relative contributions largely remain an open question.

An often-used approach to detect which community assembly processes are dominating, is the analysis of trait diversity patterns of co-occurring species (e.g. Weiher & Keddy 1995b; Cornwell & Ackerly 2009; Van der Plas et al. 2012). If community assembly is purely stochastic, one would

expect the trait composition of a local community to reflect a random subset from the regional species pool. As a result, the trait distribution of this community would be 'random'. Alternatively, if niche-based processes are important in community assembly, and the relevant traits are measured, one could expect that trait diversity of co-occurring species deviates from random. The exclusion of stress or herbivory/predation intolerant species or of competitively subordinate species (hereafter collectively called 'filtering' processes) can reduce trait diversity of co-occurring species (Weiher & Keddy 1995a; Weiher et al. 1998; Cornwell & Ackerly 2008; Mayfield & Levine 2010). On the other hand, competitive exclusion among species with overlapping niches, and exclusion of species with shared (specialist) predators (competition or apparent competition, hereafter collectively called 'limiting similarity' processes) can increase trait diversity (Hutchinson 1959; MacArthur & Levins 1967; Abrams 1983; 1999; Violle et al. 2011). By using null model comparisons, studies have tried to identify whether trait distributions differ from random and if so, whether trait composition of communities are shaped by filtering or limiting similarity processes. However, these comparisons with null models are not able to determine if such apparently random patterns are the result of true stochastic community assembly, or reflect the net outcome of opposing filtering and limiting similarity processes.

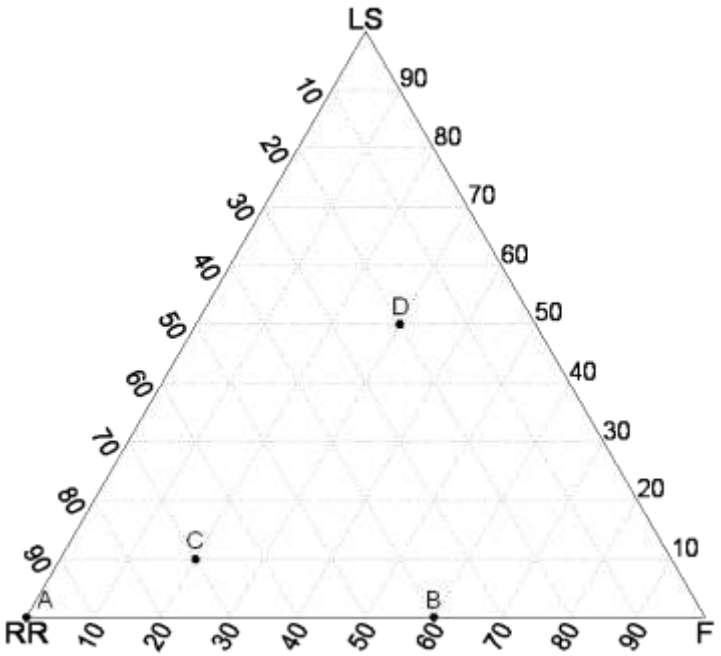


**Figure 8.1.** A ternary plot illustrating how community assembly can be seen as the sum of stochastic, filtering and limiting similarity processes. The distance till each corner represents an axis measuring to which extent community assembly is driven by stochasticity or 'random removal' (RR), filtering (F) or limiting similarity. Values of the axes range from 0 (community assembly is for 0% regulated by the given process) till 100 (community assembly is for 100% regulated by the given process). Naturally, the values of the three axes always add up to 100. The letters indicate different scenarios of community assembly: A) a community that is assembled for 100% by stochasticity; B) community assembly is for 60% regulated by stochasticity and for 40% by filtering processes; C) community assembly is for 70% regulated by stochasticity, for 20% by filtering and for 10 % by limiting similarity processes and D) community assembly is for 20% regulated by stochasticity, for 30% by filtering and for 50% by limiting similarity.

Furthermore, the relative contribution of these processes remains uncertain with these kinds of analyses, as they aim to discriminate between one assumption ($H_o$: trait distribution is random) using a two tailed approach ($H_A$: trait distribution is higher or lower than random). Despite recent improvements in the implementation of null models (e.g. Helmus et al. 2007; Cornwell & Ackerly 2009), the understanding that one can gain from these types of models is thus inherently limited.
In this study, we develop a new tool for investigating community assembly processes in observed communities, allowing the quantification of their relative importance in single communities and the analysis of trends of the importance of different processes along environmental gradients. Our basic approach consists of the stepwise removal of species from a regional species pool until a local community with certain richness is created. At each step, a species is removed through either a stochastic, filtering or limiting similarity 'event', with the relative contribution of these different events differing between alternative models (Fig. 8.1). So in fact, we study the process of 'stepwise community assembly' through the inverse process of 'stepwise species deletion', hence our name Stepwise Community Assembly Models (STEPCAM). Using Approximate Bayesian Computation (Beaumont et al. 2002; Beaumont 2010), we then compare the mean trait values and trait distributions of modeled communities with patterns of real communities to identify which overall relative importance of stochastic, filtering or limiting similarity events has most likely shaped that particular community.
To illustrate this new STEPCAM approach, we applied it to tree communities from a South African savannah. African savannahs in general (Scholes et al. 2002) and our study site in particular (Whately & Porter 1983) are known for their high habitat heterogeneity. This high habitat heterogeneity provides a major challenge for our understanding of local community assembly, because it is likely that the different communities one finds in different habitats have been shaped by different processes (Cornwell & Ackerly 2009). Other studies have shown that spatial turnover of plant communities in savannahs can often be explained by environmental gradients in rainfall (Reed et al. 2009) and fire (Higgins et al. 2007). However, how these environmental gradients regulate changes in community composition is largely unknown. Possibly, in more 'benign' (high rainfall, low fire frequency) areas, plant communities are less shaped by filtering processes and more by competition. Alternatively, it might also be that in these areas, all species that are present in the larger 'regional species pool' are also able to cope with both the local abiotic and biotic conditions, so that stochastic processes are more important in explaining community assembly.
To test our STEPCAM approach and these ideas, we characterized tree communities in 20 plots that were distributed along the main rainfall and fire gradient in the study area. Eight functionally relevant traits (e.g. specific leaf area, leaf size, wood density, leaf nitrogen and carbon content) were measured for each species and subsequently used to calculate several multidimensional trait distribution metrics for each plot. We applied our STEPCAM models and traditional null models to these data and addressed the following questions: (1) which type of community assembly processes (stochastic, filtering or limiting similarity processes) are generally most important in shaping these savannah tree communities; (2) does our community assembly model selection procedure lead to different conclusions than traditional null models and (3) how do the relative contributions of different community assembly processes change over environmental gradients?

# 8.3 Methods

## 8.3.1 Study area

Fieldwork was carried out in Hluhluwe-iMfolozi Park (HiP, 28°00'-28°26'S, 31°41'-32°09'E), South Africa. The reserve is a ~90.000 ha sized area, characterized by a high habitat heterogeneity (Whateley & Porter 1983), with as main vegetation types upland forests, savannah grassland and thickets, woodlands and riverine forests. Mean annual rainfall ranges from 500 to 1000 mm, generally increasing with altitude (Balfour & Howison 2002, van der Plas et al. 2012).At smaller scales,

heterogeneity is partially explained by variation in fire frequency, with fire return intervals ranging from 2 to 6 years, and partially by soil variation.

### 8.3.2 Tree community data

From November till December 2009, tree communities were characterized in 20 plots, stratified at different locations in HiP, covering both the rainfall and fire gradients (Fig. S8A,B). For safety reasons plots were established within 500 meter from roads and at least 500 meters away from any larger rivers. The plots measured 20 x 20 meter and were at least 550 meters away from each other, with an average nearest neighbour distance of 3.0 km. Within these plots, all individual trees taller than 0.5 meter were identified following Pooley (1997).

### 8.3.3 Trait data

We measured eight different functional traits: Leaf Area (LA), Specific Leaf Area (SLA), wood density (WD) and concentrations of leaf carbon (C), nitrogen (N), phosphorous (P), sulfur (S) and potassium (K). These traits are related to growth – longevity trade-offs, drought tolerance, nutritional status and attractiveness to herbivores (e.g. Brown & Lawton 1991; Marschner 1995; Weiher et al. 1999; Hacke et al. 2011; Westoby et al. 2002). LA, SLA, WD, leaf C and N content were measured for each species in each plot, while the other chemical traits (leaf P, S and K content) were measured for each species in up to 7 plots.

For LA measurements, for each species in each plot we collected between 5 and 30 leaves (depending on the size and weight of the leaves) from multiple individuals. The collected leaves were photographed in the field with a reference scale on the background. We used the software SigmaScanPro v 5.0 (Systat Software Inc., San José, CA) to measure LA (in $cm^2$) of fresh leaves. The leaves were dried (48h at 50°C) and dry weight was measured to 0.001 g precision. SLA (in $cm^2g^{-1}$) was calculated by dividing the LA by this dry weight. For WD measurements, at each site we collected three (20 cm, diameter between 2 and 13 mm) branches of each species. These were oven dried (48h at 50°C), dry mass was measured and we calculated WD as: $WD = \frac{\pi r^2 L}{M}$, with WD for wood density in $m^2kg^{-1}$, where $r$ is the radius of the branch in m, $L$ the length of the branch in m and $M$ the dry weight of the branch in kg. For chemical analyses, we collected bulk samples of healthy, intact leaves for each species in each site. These were brought to the HiP field station and dried (48h at 50°C). These samples were subsequently ground using a ball mill. Leaf C and N content were measured in duplicate using a Carlo-Erba NA 1500 element analyzer (Carlo-Erba, Milan, Italy). Leaf P, S and K contents were measured at the laboratory of BLGG AgroXpertus in Wageningen via ICP atomic emission spectrometry, conforming to NEN6966.

### 8.3.4 Environmental data

Rainfall data were collected at 17 more or less evenly distributed permanent rain gauge stations throughout HiP between 2001 and 2007. This data was used to calculate the annual amount of rainfall (in mm) at each gauge station location throughout this period. Using a Kriging interpolation method, we constructed an annual rainfall map for HiP, using Arc-GIS v 9.3.1 (ESRI, Redlands, California, USA) (Fig. S8A). For fire frequency, we used a digitized 200 x 200 meter resolution map with fire annually recorded between 1956 and 2004 by the park management authorities (Fig. S8B).

### 8.3.5 Calculating species and community trait averages

For each species and each trait, we calculated the Species Average Trait Value (SATV). We estimated missing SATVs using Multiple Imputation with Chained Equations (MICE) using the 'mice' function from the MICE package (Van Buuren & Groothuis-Oudshoorn 2011) in R-2.13.1 (R Development Core Team 2011). Prior to other analyses, we standardized all SATVs to a mean of zero and a standard deviation of 1. As such, we avoided that traits with higher mean or variance had higher impact on model outcomes. SATVs were used to calculate the Community abundance-weighted Trait Means (hereafter: community trait mean or CTM) for each plot: $CTM = \sum_{i=1}^{s} a_i SATV_i$, in which $s$ is

the species richness in the given plot, $a_i$ the abundance of the *i*-th species and $SATV_i$ the SATV of the *i*-th species.

### 8.3.6 Calculating Functional Diversity measures

In the past, several metrics have been developed that are aimed to capture multidimensional diversity in trait values. Here, we focus on three orthogonal metrics that capture the functional composition (or Functional Diversity - FD) of a site: Functional Richness (FRic), Functional Evenness (FEve) and Functional Divergence (FDiv) (Villéger et al. 2008). FRic was measured as the convex hull volume that can be drawn around the multidimensional trait space of all species present in a community (Cornwell et al. 2006), hence representing a multidimensional analogue to trait range. Filtering is expected to reduce FRic (Cornwell et al. 2006). For presence-absence data FEve measures the regularity of branch lengths of a minimum spanning tree in multidimensional trait space (Villéger et al. 2008), and is bounded between zero (traits are unevenly spread) and one (traits are perfectly evenly spread). FEve can thus be seen as a metric to detect limiting similarity. Lastly, FDiv was measured as the dispersion of measured species in reference to the mean community trait value, so that low FDiv represents functional clustering and high FDiv represents functional dispersion (Villéger et al. 2008). This metric can therefore be seen as an indicator of habitat filtering. FRic, FEve and FDiv were calculated for each plot and its present SATV values using the 'dbFD' function from the FD package (Laliberté & Shipley 2011) in R-2.13.1 (R Development Core Team 2011).

### 8.3.7 Null models

We developed classic null models to investigate whether FD metric values observed in each plot were higher or lower than expected by chance. For each plot, we created 1000 'randomized communities' based on a randomized sampling of the species pool found in our study (i.e. all species observed), where the chance of a species being selected was equal to its relative frequency in the region (i.e. the number of plots in which it was observed). Each randomized draw was constrained to have the same richness as the observed community.

Deviation from the null expectation ($H_0$: observed FD patterns are not distinguishable from the randomized expectation) was determined using the number of times that observed FRic, FEve and FDiv values were higher or lower than the FD measures from randomly generated (null) communities (i.e., two-sided test, $\alpha = 0.05$) (Gotelli & Graves 1996). If observed is greater than the null predictionin 975 or more randomizations (i.e. a ratio of 0.975) limiting similarity processes are regarded to determine community assembly, while if observed is greater than null in 25 or less randomizations (i.e. a ratio of 0.025), then filtering is the main process in community assembly. Hereafter, the ratio of randomizations in which lower FRic, FEve and FDiv values were found than in the associated observed communities are called 'FRic ratio', 'FEve ratio' and 'FDiv ratio' respectively. Null models were performed using R-2.13.1 (R Development Core Team 2011).

### 8.3.8 Stepwise Community Assembly Models (STEPCAM)

We developed a new modeling framework that simulates community assembly through the stepwise removal of species from a species pool, according to different removal rules, reflecting different types of processes. Starting with all the species from a regional 'species pool', each step a species is removed until the remaining set of species reaches the same species richness as the 'local community' under investigation. Therefore, each simulation model contains *n* steps, in which *n* equals the species richness of the species pool (*S*) minus the species richness of the local community (*s*) (Fig. 8.2). The species pool is defined as all tree species across all 20 plots sampled, with overall species richness (*S*) of 105. Richness in our local communities (*s*) varied from 5 to 27, with an average of 14.4 species. Thus, the number of steps in our simulation models varied from 100 (= 105 – 5) to 78 (= 105 – 27).

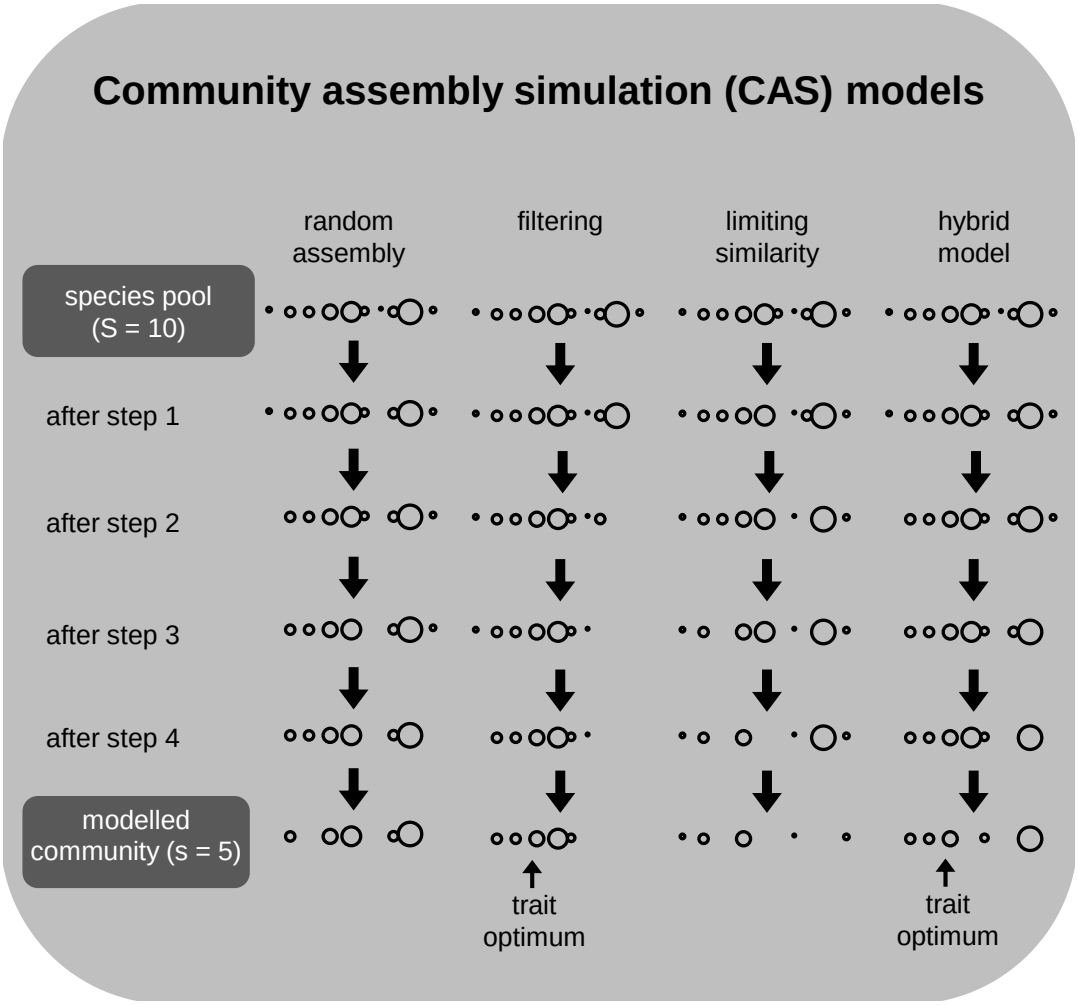


**Figure 8.2.** An illustration of four different CAS models. These four models differ in their parameter settings, with from left to the right, (i) a model only containing random removal steps, (ii) a model only containing filtering steps, (iii) a model only containing limiting similarity steps and (iv) a model consisting of 2 (i.e. 40 %) random removal steps, 1 (i.e. 20 %) filtering step and 2 (i.e. 40 %) limiting similarity steps. In this simple example, each model starts with the same species pool, containing S = 10 species. Species are represented by circles, in which the size of the circle is proportional to its frequency in the species pool and its horizontal location reflects its trait value. For simplicity, we in this example we focus on a one-dimensional trait space in this example. We set the richness (s) in the local community to 5 species, so that each model consists of S – s = 5 steps. Each step, a species is removed, either through (1) random removal, in which species that occur more frequently in the species pool have a lower chance of being removed, (2) filtering, in which the species farthest from the trait optimum (arrow below) is removed, or (3) limiting similarity, in which the species most similar to its neighboring species is removed.

During each step, a species is removed following either one of three different mechanisms: (1) random removal, (2) filtering or (3) limiting similarity (Fig. 8.1). For each plot a series of competing STEPCAM models were generated, by changing the proportion of steps in which each of the three evaluated mechanisms are applied. In each model, first the random removal steps were run, then the filtering steps, and finally the limiting similarity steps. We thus assumed a certain order in community assembly, which is in line with other literature on this topic (e.g. Cornwell & Ackerly 2009; Van der Plas et al. 2012). As a robustness check for the method, we investigated whether this order of different community assembly steps highly altered FD measures, but this was not the case (Supplement S8.2).

During a 'random removal' step, a species is removed from the until that point remaining set of species, with the 'removal chance' of each species being proportional to its relative frequency (i.e. the number of plots in which it was found) in the study area (Fig. 8.2). Random removal steps can thus be seen as a stochastic process in community assembly such as dispersal limitation, as emphasized in neutral or neutral-like biodiversity theories (e.g. Caswell 1976; Sale 1977; Hubbell 2001).

During a 'filtering' step, the species with an SATV value with the largest Euclidean trait distance from the 'trait optima' of the community under investigation is removed from the remaining species set (Fig. 8.2). Assuming that observed sets of species are best adapted to the local environment in which they occur and that their traits reflect these adaptations, we define trait optima as the set of CTM values from the plot under investigation. Filtering steps, which are based on Kraft et al. (2007), can thus be seen as a niche-based process in community assembly, in which species without the right adaptations are unable to occur in a certain abiotic (Weiher & Keddy 1995a; Weiher et al. 1998; Cornwell et al. 2006) or biotic (Mayfield & Levine 2010) environment.

During a 'limiting similarity' step, from the remaining species set, we first select the species pair that was most similar in SATVs, i.e. most close to each other in the multi-dimensional trait space. From this species pair, we remove the species that was closest to a third species (Fig. 8.2). This step, adapted from Kraft et al. (2007), can thus be seen as the exclusion of species with shared resources (Hutchinson 1959; MacArthur & Levins 1967; Abrams 1983; Violle et al. 2011) or predators (Abrams 1983; 1999).

To explore how FRic, FEve and FDiv values respond to different relative contributions of random removal, filtering and limiting similarity steps in our STEPCAMs, we applied several STEPCAMs, differing in these parameter settings, to plot nr. 17. This plot was considered representative due to its annual amount of rainfall, fire frequency and species richness values, which were close to the average values across all 20 plots (Fig. S8A,B). In each different STEPCAM, the relative contributions of random removal, filtering and limiting similarity steps add up to 1. We explored this parameter space with 0.05 intervals, so running models with parameter settings of 1-0-0, 0.95-0.05-0, 0.95-0-0.05, 0.9-0.1-0, 0.9-0.05-0.05, etc., in which the first number stands for the relative contribution of random removal steps in the model, the second number for the relative contribution of filtering steps and the last number for the relative contribution of random removal steps. Consequently, we ran 231 (= $\frac{(\frac{1}{0.05}+1)\cdot(\frac{1}{0.05}+2)}{2}$) different models, with 1000 replicates for each one. For each species set resulting from a CAS model, we calculated FRic, FEve and FDiv and we calculated the average of the 1000 replicate FRic, FEve and FDiv for of the 231 different STEPCAMs. The STEPCAMs were run in R-2.13.1 (R Development Core Team 2011).

### 8.3.9 STEPCAM Model fitting

Model fitting was performed through Approximate Bayesian Computation (ABC) within a Sequential Monte Carlo (SMC) framework (Toni et al. 2009; Hartig et al. 2011). In ABC one or more summary statistics of observed data are compared with those generated by models. This is in contrast to Maximum Likelihood optimization where the likelihood is used to find the best fitting model. For our STEPCAMs no likelihood could be calculated and therefore we relied on the use of four summary statistics: FRic, FEve, FDiv and CTM values. Before model selection, we standardized all summary statistics to a mean of zero and a standard deviation of one, so that each different summary statistic had the same impact on the fitting procedure. The fit of STEPCAMs was calculated as:

$$Fit_{total} = Fit_{FRic} + Fit_{FEve} + Fit_{FDiv} + Fit_{CTM},$$

in which $Fit_{FRic}$, $Fit_{FEve}$ and $Fit_{FDiv}$ are the absolute difference between respectively FRic, FEve and FDiv values from the observed community and those generated by the STEPCAM, while $Fit_{CTM}$ is the (multidimensional) Euclidian distance between the CTMs of the observed community and those generated by the CAS model. High $Fit_{total}$ values thus indicated poor fit, while low values indicated good fit. Using the fit of the models, importance resampling was performed largely following the algorithm described in Toni (2009). For more detailed information on the algorithm we used, see S8.3. For each plot, we ran the algorithm 10 times for different starting values, to investigate whether the ABC model selection generated the same parameter values (S8.4). The average parameter values of these 10 models are reported in the 'results' section.

To investigate whether our ABC-SMC approach could adequately distinguish between competing STEPCAMs, we used a reverse fitting procedure: we ran STEPCAMS for plot 17 with known parameter values (the 'generator models'), generating summary statistics. These generated summary statistics were fitted with the ABC-SMC approach described above to select 'best fitting models'. Comparing parameter values of the 'generator models' and of the 'best fitting models' then provides valuable insight in the power of our ABC-SMC approach: if our approach is perfect in distinguishing different STEPCAMs, parameter values of 'generator models' and 'best fitting models' should ideally be identical. For a more specific description of this reverse fitting procedure, see S8.4.

### 8.3.10 Relationships between tree community assembly and environmental gradients

Using general linear models (GLMs), we investigated the relationships between FD ratio values (Null model outcomes) and environmental gradients. We first created full models, containing annual rainfall, fire frequency, species richness and log abundance as predictor variables for FRic ratio, FEve ratio and FDiv ratio, and rainfall and fire frequency as predictor variables for species richness, log abundance and CTM values. We then ran a Maximum Likelihood model-selection procedure, identifying the model with the lowest AIC value only containing significant predictor variables. Relative contributions of random removal, filtering and limiting similarity steps in best fitting STEPCAMs were not independent from each other, and were therefore analyzed simultaneously, using Multivariate Analysis of Variance (MANOVA). Fire, rainfall and their interaction effect were used as predictor variables for the MANOVA.

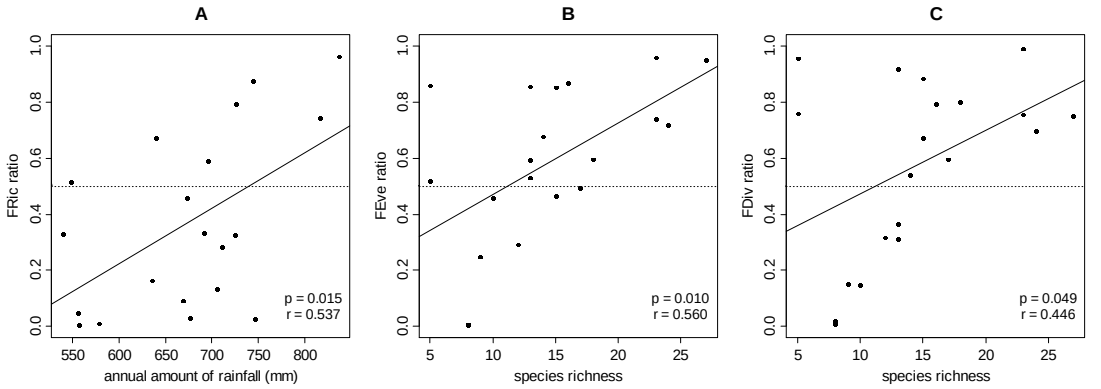


**Figure 8.3.** Relationships between Functional diversity metrics and rainfall / species richness. (a) Relationship between FRic ratio and annual amount of rainfall (mm) ($R^2$ = 0.288). (b + c) Relationship between FEve ratio and FDiv ratio respectively and species richness ($R^2$ = 0.313 and 0.199 respectively). The dotted line shows were FRic ratio, FEve ratio and FDiv ratio values are exactly as high as expected by chance.

## 8.4 Results

### 8.4.1 STEPCAMs

In line with expectations (Cornwell et al. 2006), FRic was lowest in species assemblages which were mostly formed by filtering steps and highest in species assemblages that were mostly formed by limiting similarity (Fig. 8.4, see also Fig. 8.1 for how to read the ternary plot). FEve and FDiv were low in communities completely shaped by filtering or random removal steps, and higher in communities mostly formed by limiting similarity steps (Fig. 8.4). However, FEve and FDiv values were even higher in species assemblages that were partially formed by filtering steps and partially by limiting similarity steps (Fig. 8.4).

For most plots in our dataset, best fitting STEPCAMs had a high relative contribution of random removal steps (average across plots: 76.1 %), a lower relative contribution of filtering steps (average across plots: 18.2 %) and even fewer limiting similarity steps (average across plots: 5.7 %) (Fig. 8.5). Reverse fitting of the trait patterns resulting from 'generator' STEPCAMs with known parameter settings (i.e. known relative contributions of different community assembly processes) showed that the fitting procedure was relatively robust: on average, parameter settings of the 'generator' models hardly differed from their associated 'best fitting' models selected using the ABC-SMC approach (S8.4, Fig. S8D). Average parameter deviations between 'best fitting' models and associated 'generator' models were 3.5 % for random removal, 2.8 % for filtering and 1.8 % for limiting similarity, which was much lower than the 27.7 % deviation that one would expect for each parameter value if the ABC-SMC approach would be completely uninformative (Fig. S8D). This check thus suggests that the parameter settings of the models best fitting the 20 observed communities are reliable.

The MANOVA showed that the relative contribution of the different STEPCAM steps responded significantly to variation in annual amount of rainfall and fire frequency, with limiting similarity steps having a higher contribution in plots with high rainfall, especially when fire frequency was low (Table 8.1; Fig. 8.6).

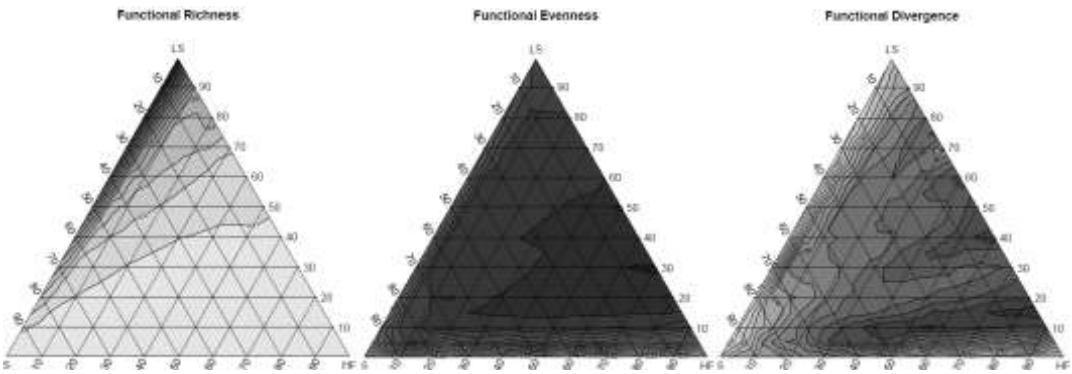


**Figure 8.4**. Ternary plots with the responses of FRic (a), FEve (b) and FDiv (c) to changes in the relative contribution of random removal, filtering and limiting similarity steps in CAS models applied to plot 17. Dark colours indicate high FD values, light colours indicate low FD values. FD values were calculated for 231 different CAS models. FD values for other positions in parameter space were estimated using bilinear interpolation. Abbreviations: RR = random removal, F = filtering and LS = limiting similarity.

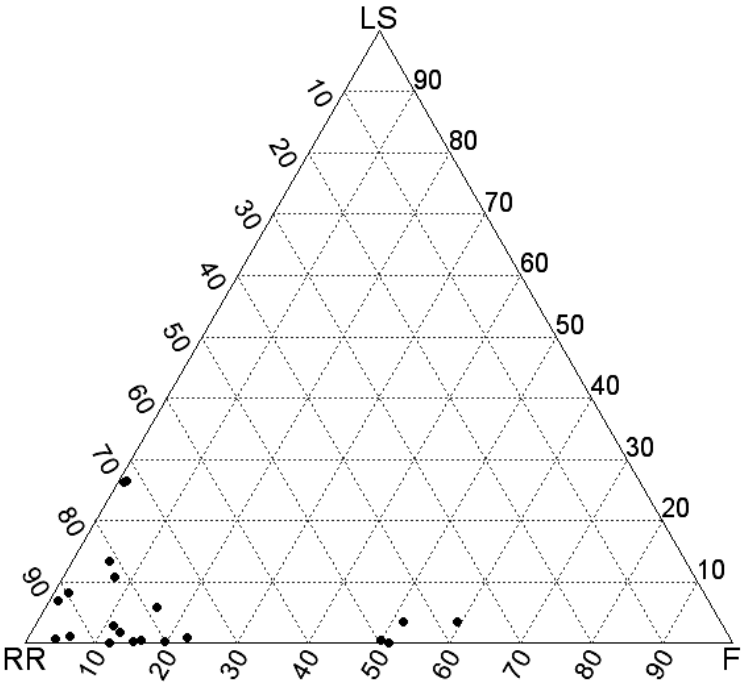


**Figure 8.5.** Ternary plot with the dots representing the best fitting CAS model for each of the 20 plots. Abbreviations: RR = random removal, F = filtering and LS = limiting similarity.

### 8.4.2 Null models

In 3 plots, FRic was lower than expected by chance, while lower FEve and FDiv values than expected by chance were found in 2 plots. FRic, FEve or FDiv values significantly higher than expected by chance were never found. FRic ratio increased with annual amount of rainfall (Table 8.1), with FRic tending to be lower than random in plots with low rainfall, while tending to be higher than random in plots with high rainfall (Fig. 8.3). Possibly this was because of constrained (low) LA and (high) WD values in low rainfall plots (Table 8.1). FEve and FDiv ratio were highest in plots with highest species richness (Table 8.1), with FEve and FDiv tending to be as high as expected by chance in plots with low species richness, but higher than expected by chance in plots with higher richness (Fig. 8.3).

### 8.4.3 Relationships between tree richness and density and environmental gradients

Tree species richness decreased with an increasing frequency of fires (Table 8.1), with average richness going down from approximately 19 species in plots with lowest fire frequency to approximately 9 in plots with highest fire frequency (Fig. 8.6). Tree abundances did not respond to fire frequency or annual rainfall (Table 8.1).

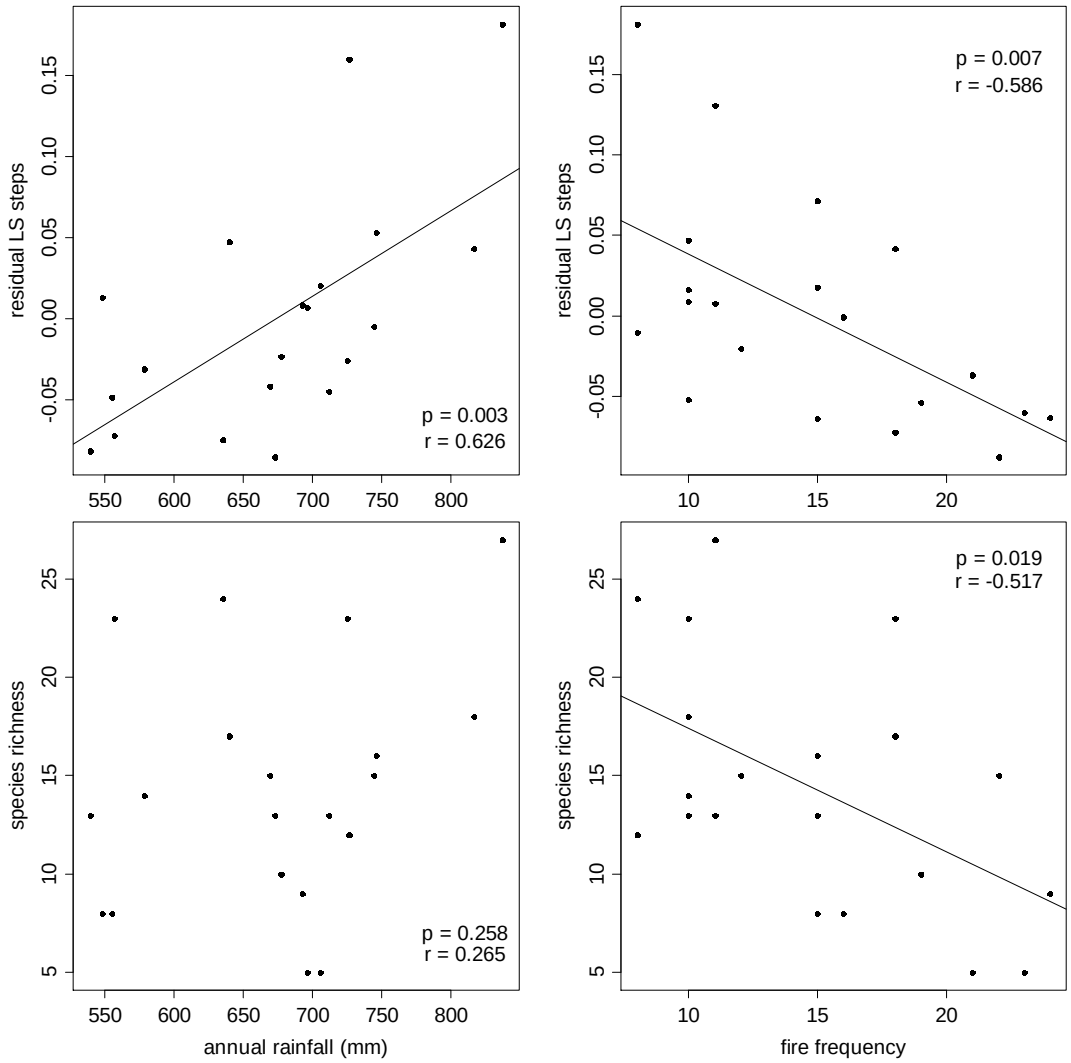


**Figure 8.6.** Relative contribution of limiting similarity (LS) in best fitting CAS models vs. annual amount of rainfall (left) and fire frequency (right) (up) and species richness vs. annual amount of rainfall (left) and fire frequency (right) (down). Note that in the upper graphs, on the y-axes residual values are shown, from general linear models with for the relative contribution of limiting similarity vs. fire frequency (left) and vs. rainfall (right).

**Table 8.1**. GLM and MANOVA (for CAS model results) outcomes. Significant predictor variables in best fitting GLMs are given.

| Response variable | Variables in final model with *T* or *F* and *P* value | $R^2$ |
|---|---|---|
| **Diversity and density** | | |
| Species richness | Fire frequency (*T* = -2.560; *P* = 0.0197) | 0.267 |
| Log abundance | | 0.000 |
| **CTM values** | | |
| SLA | | 0.000 |
| LA | Rainfall (*T* = 5.236; *P* < 0.0001), fire frequency (*T* = -4.033; *P* = 0.0009) | 0.695 |
| WD | Rainfall (*T* = -2.428; *P* = 0.0259) | 0.247 |
| C content | | 0.000 |
| N content | Fire frequency (*T* = 2.613; *P* = 0.0176) | 0.275 |
| P content | | 0.000 |
| S content | | 0.000 |
| K content | | 0.000 |
| **Null models** | | |
| FRic ratio | Rainfall (*T* = 2.698; *P* = 0.0147) | 0.288 |
| FEve ratio | Species richness (*T* = 2.866; *P* = 0.0103) | 0.313 |
| FDiv ratio | Species richness (*T* = 2.111; *P* = 0.0490) | 0.199 |
| **CAS model outcomes** | | |
| Random removal | Rainfall (*T* = -0.905; *P* = 0.379), fire frequency (*T* = -0.911; *P* = 0.376), rainfall x fire frequency (*T* = 0.895; *P* = 0.384) | 0.053 |
| Filtering | Rainfall (*T* = 0.009; *P* = 0.993), fire frequency (*T* = 0.308; *P* = 0.762), rainfall x fire frequency (*T* = -0.225; *P* = 0.825) | 0.108 |
| Limiting similarity | Rainfall (*T* = 3.047; ***P* = 0.008**), fire frequency (*T* = 1.975; *P* = 0.066), rainfall x fire frequency (*T* = -2.223; ***P* = 0.041**) | 0.635 |

# 8.5 Discussion

For each of the 20 savanna tree communities investigated during this study, we used our STEPCAMs and the ABC model selection approach to estimate the relative contributions of stochasticity, filtering and limiting similarity processes in community assembly. Before focusing on our main results, we first discuss the robustness of our novel method. Several authors have suggested that community assembly processes are very difficult to separate when operating simultaneously, because effects of filtering and limiting similarity on trait distribution patterns are expected to be opposite (Kraft et al. 2007; Weiher et al. 2011; Butterfield & Callaway 2013). However, our reverse fitting procedure of patterns generated by particular target models showed that we could successfully distinguish different community assembly scenarios with varying importance of different processes (S8.4). Additionally, when fitting the models to real data, the posterior parameter distribution generated by our SMC procedure was reasonably narrow, showing that our STEPCAM models generated repeatable trait distribution patterns. We believe that simultaneously fitting several summary statistics (Fric, Feve, Fdiv and CTM values) that are relatively independent from each other (Villéger et al. 2009) contributed to the robustness of the modeling procedure.

When applying our method, we found that the best fitting models consisted mostly of stochastic or random removal steps (on average 76.1 %), less of filtering steps (on average 18.2 %) and least of limiting similarity steps (on average 5.7 %). Stochastic processes thus seem to be the most important

community assembly processes in this savannah ecosystem. However, it should be emphasized that contrary to assumptions in purely neutral models (e.g. Hubbell 2001), stochasticity was *not the only* process shaping communities – it was the dominant one.

An interesting question is why in a system with some very clear constraints on tree recruitment and growth (due to e.g. drought, fires and herbivory), stochasticity is so important for community assembly. Part of the answer lies in our definition of the species pool, which only included species found in our set of 20 plots. Therefore, larger scale filtering processes that determine which species can (e.g. species able to tolerate fires / herbivory) or cannot (e.g. species unable to tolerate fires / herbivory) occupy any of our plots are ignored in this study. Nonetheless, *within* our study design, there are still clear environmental gradients (see Fig. S8A,B) and the question remains why tree communities sampled over these gradients appeared relatively randomly assembled. One reason might be that although there are some very clear disturbances or stressful conditions limiting tree performance, species are rather similar in coping with such disturbances or stressful events. Species can have very different strategies to cope with the same kind of environmental conditions resulting in contrasting phenotypes with similar fitness (Marks & Lechowicz 2006). For example, in savannas, some plant species survive in an environment with high herbivore densities because of adaptations to avoid herbivory (spines, tannins, alkaloids), while others tolerate herbivory instead (Bryant et al. 1989). Also, some traits can serve multiple functions. For example, thick wax layers on leaves and spines can defend a plant against herbivory, but can also make it drought resistant. As a result, organisms of different species could become functionally equivalent (Hubbell 2001), despite strong differences in traits, leading to emergent neutral trait distribution patterns.

Filtering, which seemed a more important community assembly process than limiting similarity, but less important than stochasticity, is most often interpreted as the exclusion of species that are intolerant to some common (a)biotic stress factor. Drought, fires and browsing are commonly recognized stress factors for savanna trees (e.g. Walter 1971; Bond & Wilgen 1998; Scholes et al. 2002) and may all have been responsible for the imprints of filtering on trait distribution patterns of tree communities. Additionally, filtering patterns might also be caused by the exclusion of competitively inferior species (Mayfield & Levine 2010). For example, in the wettest plots, fast growing species with low wood density values might have outcompeted slow growing species with high wood density values.

Although our selected models generally contained only a very small proportion of limiting similarity steps, their contribution became higher in plots with high rainfall and low fire frequency. Limiting similarity is usually interpreted as an indicator of interspecific competition (Hutchinson 1959; MacArthur & Levins 1967; Abrams 1983; Cornwell & Ackerly 2009). In savannas, were tree growth is limited by water availability and fires (e.g. Bond & Wilgen 1998; Scholes et al. 2002) and canopies are quite open, light competition among trees is in most cases likely to be modest (van der Plas et al. 2013). Those sites with low fire frequency and a relatively high amount of annual rainfall (a rare combination) may be among the few locations where light competition among trees is strong enough to detect significant impacts of limiting similarity. Interestingly, these were also the sites with highest species richness. This suggests that perhaps, richness was highest because other factors than limiting similarity (filtering, stochasticity) were relatively important in excluding species.

It is likely (as in any study of this type) that our decisions on which traits to include have affected our overall conclusions. We included traits that were mostly related to stress tolerance – growth rate trade-offs or to herbivory. We did not study traits related to reproduction (such as seed size) or to water and nutrient uptake strategies (such as rooting depth or mycorrhizal associations). If such traits would have been included, this might have led to a relatively lower contribution of stochasticity in selected models, and a higher contribution of niche-based processes. This is a general point in the trait-based community assembly literature: conclusions are only valid *given* the set of selected traits. We should thus emphasize that based on our simulation models and our selected traits, community assembly is thus relatively highly stochastic *with respect to* the specific traits that we measured.

Also, choices about which type of community (functional group, guild) to study and its delineation is expected to affect the outcome of the type of study that we performed. Here, we studied tree

communities. Savanna trees do not only interact with each other, but also with the grass layer (Cramer et al. 2010) and one could thus expect that while there is no strong evidence for limiting similarity within tree communities, trees might compete more strongly with other plants. So if the plant community of the savannah was defined as trees, grasses and forbs together, we might have made different conclusions on the importance of different processes.

Lastly, inclusion of different processes or different levels of taxonomic aggregation in our models might have led to different outcomes. In our models, we did not take intraspecific trait differences into account and additionally, there are ecological processes, such as facilitation and mutualisms, which are not explicitly included. Therefore, future model modifications to study additional effects of intraspecific trait variation (as suggested by Siefert 2012) or other ecological processes (as suggested by Butterfield & Callaway 2013) on community assembly may be fruitful.

When comparing the outcomes of our STEPCAM approach with null model outcomes, one main finding is that while null models never detected any sign of limiting similarity in our dataset, our STEPCAM approach did. Limiting similarity and filtering are expected to have more or less opposite effects on the distributions of traits of co-occurring species (Weiher et al. 2011) and therefore the impact of one of these processes can be masked by the other. Although other studies have suggested solutions for these problems (e.g. Helmus et al. 2007; Cornwell & Ackerly 2009), it remains an open question whether these work in under all circumstances. We have shown that the traditional assumption that filtering decreases trait diversity, while limiting similarity increases it, is a highly simplified one, as the interaction of these processes causes nonlinear patterns in parameter space (Fig. 8.4). Therefore, when limiting similarity processes and filtering processes operate simultaneously, at similar spatial scales, with null models it may be very hard to statistically detect both these processes and even impossible to quantify the relative contributions of these different processes to community assembly. With our STEPCAM approach, we have mostly overcome these problems. We therefore believe that our approach is promising to unravel the relative importance of different community assembly processes across different types of ecosystems, types of communities, trophic levels and global environmental gradients.

## 8.6 Acknowledgements

We thank Nelly Eck for doing part of the chemical analyses. We also thank Francisco Encinas Viso and Adriana Alzate Vallejo for useful discussions about this study.

# Section IV: Synthesis

# Chapter 9: Inferring the presence and relative importance of different community assembly processes from the distribution of functional traits: a synthesis

Fons van der Plas

## 9.1 Introduction

Biodiversity is declining in almost all ecosystems on earth (Stuart et al. 2004; Sala & Knowlton 2006; Butchart et al. 2010) and there is general consensus that most present day species extinctions are caused by humans (Stuart et al. 2004; Sala & Knowlton 2006; Sala & Knowlton 2010). This biodiversity decline takes place at several spatial scales: globally, regionally, but also at the local scale of communities (Sala & Knowlton 2006). While global species extinctions usually raise more concerns to the general public than local ones, they cannot be understood without insight in the latter. Indeed, global species extinctions are only the final step of a very long sequence of local extinctions. This makes understanding how local communities of co-occurring species are formed one of the most challenging goals in ecology.

'Community assembly theory' is the field focusing on the 'processes' or 'rules' forming ecological communities. Although preceeded by some initial attempts (e.g. Clements 1916), Jared Diamond is often seen as the pioneer of the field. He introduced the term 'community assembly' after discovering that certain bird species on islands near New Guinea never co-occurred, and attributed these 'forbidden species combinations' as the result of competitive exclusion (Diamond 1975). Although the basic debate on the role of chance versus competition was quickly picked up (Connor & Simberloff 1979), it took until the nineties before the study of community assembly became popular among more ecologists (e.g. Drake 1991; Weiher et al. 1998). In the following decades, the study of community assembly became an established and increasingly popular subfield of ecology (Fig. 9.1).

Most practitioners focused their research on two main ecological, niche-based processes driving community assembly: 'abiotic filtering' in stressful environments (Box 1) and 'limiting similarity' of shared resource acquisition (i.e. resource competition) (Weiher & Keddy 1995a; Weiher et al. 1998; Webb et al. 2002; Cavender-Bares et al. 2004). Reasoning that 'functional traits', such as growth rate, seed size or height (for plants), or metabolic rate, dispersal capability or diet (for animals) mediate such niche-based processes (Weiher & Keddy 1999; McGill et al. 2006), trait data have often been applied to detect these.

In the absence of niche-based ecological processes, the distribution of species traits in a local community is expected to reflect a random subset from the traits of a larger (more regional) species pool. However, in habitats with severe, stressful conditions, species without the right traits are usually expected to be unable to survive or reproduce, resulting in an assemblage of co-occurring species sharing very similar traits (hereafter called trait underexpansion, Fig. 9.2, see also Box 1). This process is usually termed environmental or abiotic filtering (Box 1) (Weiher & Keddy 1995a; Cavender-Bares et al. 2004). On the other hand, if the competitive exclusion of species sharing similar resources is the most important process in shaping communities, species traits in resulting assemblages are usually expected to be rather dissimilar from each other (hereafter: trait overdispersion, Fig. 9.2, see also Box 1), a process called limiting similarity (Hutchinson 1959; Kraft et al. 2007; Cornwell & Ackerly 2009). Typically, null models have been used to investigate whether trait distribution patterns (see Box 1) in communities are either underexpanded, overdispersed, or not different from random (e.g. Weiher & Keddy 1995a; Cavender-Bares et al. 2004; Kraft et al. 2008; Cornwell & Ackerly 2009).

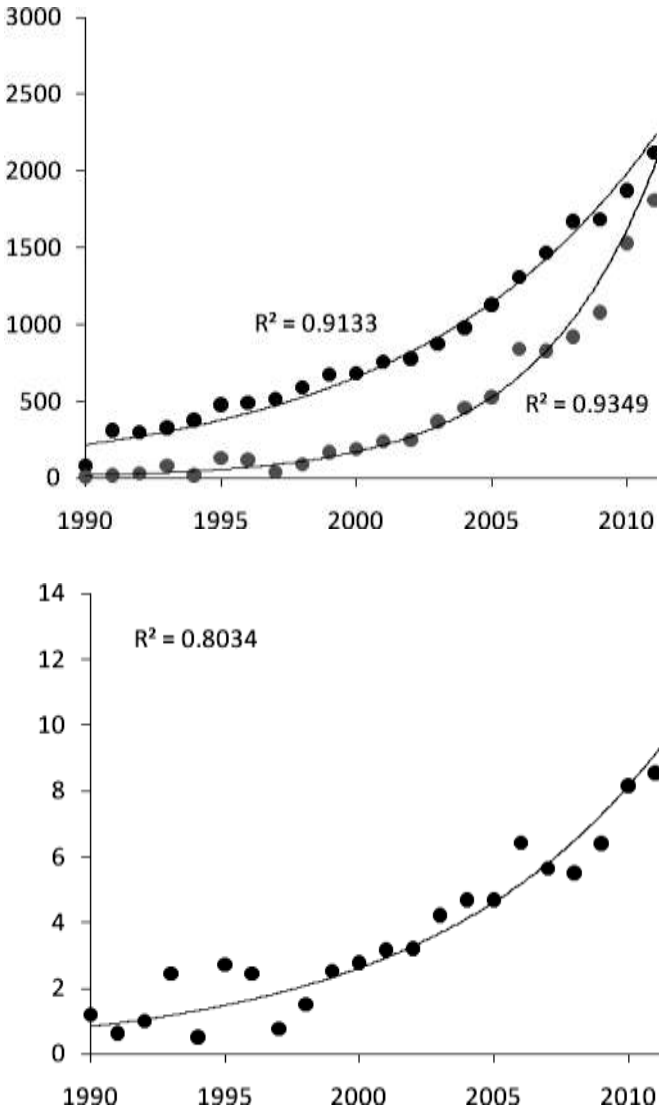


**Fig. 9.1.** The number of publications in the period of 1990-2012 found in the Web of Science database and accessible to the University of Groningen found with the key words 'communit* AND ecology' (A, dark grey dots) and found with the key word 'communit*-assembl*' AND ecology, *multiplied by ten* (A, light grey dots). Publications on both community ecology and the subfield community assembly increased exponentially in this period, although the publications on community assembly increased relatively more rapidly, with the proportion (in %) of publications containing the key words 'communit*' AND which also contain the key word 'communit*-assembl*' increasing exponentially over time (B).

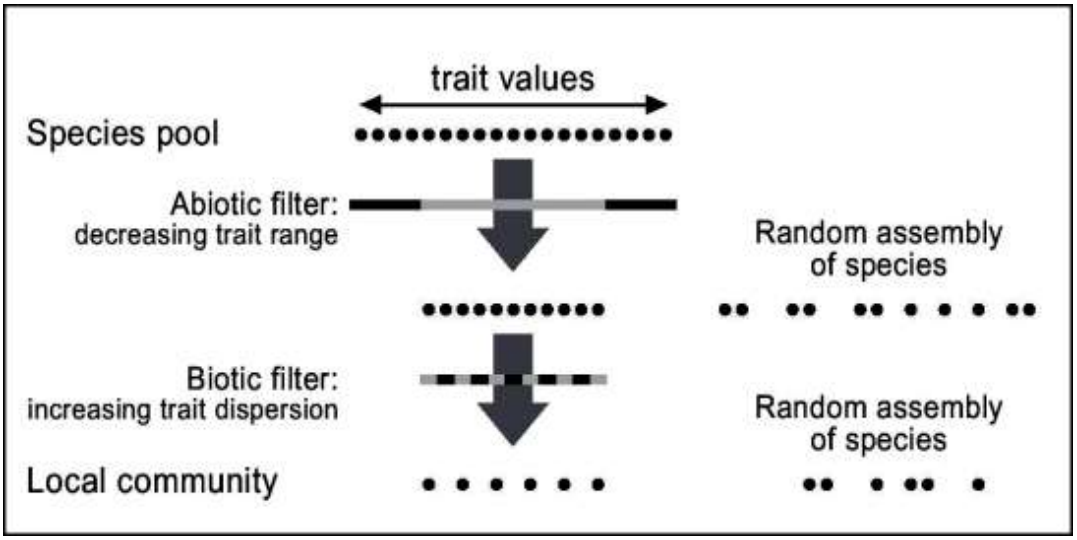


**Fig. 9.2.** A classical idea of community assembly. Here, when abiotic filters drive community assembly, only species with quitable traits are able to live in a local environment, leading to trait underexpansion patterns. 'Biotic filters', often used to describe competition, should lead to limiting similarity of shared resources between co-occurring species, and therefore to trait overdispersion patterns.

Probably due to its intuitive simplicity, this trait-based approach for studying community assembly became increasingly popular, especially over the last decade. This resulted in both an absolute and a relative increase in the number of publications on community assembly per year (Fig. 9.1). These studies have not been without reward, but led to some interesting insights. For example, while species richness and abundance distribution patterns suggest that trees in tropical rainforests are neutrally assembled (Hubbell 2001), analyses on trait distribution patterns from similar tree communities in tropical forests usually reject random community assembly (Kraft et al. 2008; Uriarte et al. 2010; Paine et al. 2011). Instead, they point at the importance of abiotic filtering (Kraft et al. 2008; Uriarte et al. 2010; Paine et al. 2011) and competition (Kraft et al. 2008) in shaping these tree communities. Additionally, experimental studies show that while community assembly at the level of species is often rather stochastic, in most cases, more deterministic processes can be identified when analyzing trait patterns (e.g. Fukami et al. 2005; Helsen et al. 2012, Box 2).

However, despite the popularity and merits of this trait-based approach in community assembly, it has also been highly criticized. These criticisms focus on the fact that the study of community assembly has become an increasingly isolated subfield in ecology, ignoring important insights from other subfields. Firstly, assumptions of how abiotic filtering and competition affect within-community trait distributions are not always in line with conceptual and mathematical models. Secondly, most studies on community assembly only focus on two niche-based ecological processes (abiotic filtering and competition), ignoring the various other types of ecological (e.g. trophic interactions, facilitation, ecosystem engineering) and evolutionary processes involved. Taking these issues into account leads to a more complete view on trait-based community (Fig. 9.3) than the traditional one (Fig. 9.2). A third issue is that although most studies try to identify which types of ecological processes have been 'present' or 'most important' in forming observed communities, in reality, communities are usually shaped by a combination of various processes, and it is still the question how the interplay of different community assembly processes affects within-community trait distributions.

In the following sections, I will discuss recent studies demonstrating the implications of these three issues. I will then discuss how humans disturb 'natural' community assembly and discuss what community assembly theory can teach conservation managers to deal with these problems. Finally, I will briefly summarize the state of the field and make recommendations for the emphasis of future research on trait-based community assembly.

**Box 9.1: Glossary**

**abiotic filtering**: a niche-based community assembly process, in which species without the right traits for coping with local abiotic conditions (e.g. drought, temperature) are excluded from living in the community. Without any opposing processes, abiotic filtering should lead to trait underexpansion patterns in communities (e.g. Weiher et al. 1998; Cavender-Bares et al. 2004)

**filtering**: a niche-based community assembly process, in which species without the right traits for coping with both abiotic (e.g. drought, temperature) *and* biotic (e.g. dominant competitor species, generalist predators) conditions, are excluded from living in the community. Note that filtering processes include, but are not constricted to, *abiotic* filtering processes. Without any opposing processes, filtering should lead to trait underexpansion patterns in communities (**chapter 7**).

**trait dispersion patterns**: the degree to which extent the trait values of all species that are present in a local community are evenly spread. Commonly used measures are: trait evenness (e.g. Cornwell & Ackerly; **chapter 3**) or the multidimensional functional evenness (Villéger et al. 2008; **chapter 1, 7**).

**trait distribution patterns**: a broad term for any kind of distribution of trait values of all species that are present in a local community. Examples of univariate measures are: trait range and evenness (Cornwell & Ackerly; **chapter 3**) and minimal trait distance (Weiher et al. 1998). Examples of multivariate measures are: functional richness, evenness and divergence (Villéger et al. 2008; **chapter 1,7**) and functional dispersion (Laliberté & Legendre 2010).

**trait expansion patterns**: the (multidimensional) range of the trait values of all species that are present in a local community. Commonly used measures are: trait range (e.g. Cornwell and Ackerly; **chapter 3**) or the multidimensional functional richness (Villéger et al. 2008; **chapter 1, 7**).

**trait overdispersion**: higher trait dispersion (i.e. a more even spread of within-community species trait values) that what one would expect if communities were assembled randomly. Trait overdispersion is usually seen as the result of limiting similarity for shared resources or predators (e.g. Weiher et al. 1998; Cavender-Bares et al. 2004; **chapter 7**).

**trait underexpansion**: lower trait expansion (i.e. a higher range or volume of within-community species trait values) that what one would expect if communities were assembled randomly. Trait underexpansion is usually seen as the result of (abiotic) filtering (e.g. Abrams 1983; Weiher et al. 1998; Cavender-Bares et al. 2004; **chapter 7**).

**Box 9.2. Responses of species and trait convergence to three main types of community assembly processes.**

If community assembly is purely stochastic, then by definition one would expect that similar sized communities sharing the same regional species pool would not look more similar to each other than expected by chance. In contrast, if community assembly is 100% driven by deterministic, niche-based processes, then in different localities sharing the same species pool and the same environmental conditions one would expect exactly the same species composition. However, communities are usually assembled by a combination of different processes (**chapter 9**), and this raises the following questions: (1) to which extent does species composition converge to a certain state under other scenarios (i.e. when both neutral and niche-based processes are driving community assembly); and (2) does trait composition has a greater tendency to converge to a certain state than species composition? Answering the first question gains our insight in how predictive we should expect species community assembly to be, while the latter should tell us whether traits data lead, as often suggested (e.g. McGill et al. 2006) to more accurate predictions on community assembly than trait data.

To answer these questions, I applied the community assembly simulation (CAS) models introduced in **chapter 9** to plot nr 17 from the same chapter. Under 231 different scenarios of community assembly (see **chapter 9**), I run these models with 20 replicates and investigated to which extent these replicates converged / diverged in both species and trait composition.

Following Helsen et al. (2012), species and trait convergence / divergence were calculated with the Bray Curtis distances in the plot x species and plot x average trait value matrices. These distance values were then standardized: all distance values were divided by the divergence value from the CAS models with 100% random assembly (i.e. all model steps are random removal), so that these neutral models were assigned a divergence value of 1, while communities that were 100% assembled by deterministic, niche-based processes (i.e. filtering and/or limiting similarity processes) had a divergence value of zero.

I then also investigated for each of the 231 CAS scenarios, whether species and trait divergence was significantly lower than expected for a neutral model, i.e. than expected for models with only random removal steps. To do this, for each of the 190 divergence values ($= \frac{(20-1)\cdot 20}{2}$) of each CAS scenario, I investigated whether this value was higher than in the neutral model. If this happened in more than 5% of the cases ($\alpha = 0.05$, one sided test), species or trait convergence in the alternative model was not significantly higher than expected at random, while in the alternative case, it was.

As expected, in CAS models only containing niche-based steps, community assembly was 100% deterministic, with both species and trait community composition converging to a certain state (Fig. B2A). In CAS models with only random removal steps, both species and trait convergence were much lower. In models with both niche- and neutral based processes, both species and trait convergence increased with the relative contribution of niche-based processes, especially with filtering steps (Fig. B2A). Interestingly, trait convergence increased much more rapidly with the relative contribution of filtering steps than species convergence did (Fig. B2A). As a result, the CAS models predict that under certain conditions (in general: random removal steps > filtering steps > limiting similarity steps), trait convergence differs significantly from random, while species convergence does not (Fig. B2B). In some other cases, when communities are hardly assembled by filtering processes, but relatively highly by random removal and limiting similarity steps, the opposite pattern can be expected (Fig. B2B) of species convergence, but no trait convergence. While these results thus suggest that a pattern of trait convergence, but no species convergence, can only be expected in some conditions (roughly: random removal steps > filtering steps > limiting similarity steps), real communities do often seem to meet these criteria: experimental studies have shown support for the trait, but not species convergence idea (Fukami et al. 2005; Helsen et al. 2012), while results of **chapter 9** also suggest that most communities meet the random removal steps > filtering steps > limiting similarity steps criterion.

**Box 2 (continued)**

One thing that we should take in mind is that when community assembly is not 100% caused by niche-based processes, both species and trait divergence are above 0 (indicating maximum convergence) (Fig. B2A). Therefore, we will never be able to make 100% reliable predictions on this topic, neither in case with species nor in case with trait data.

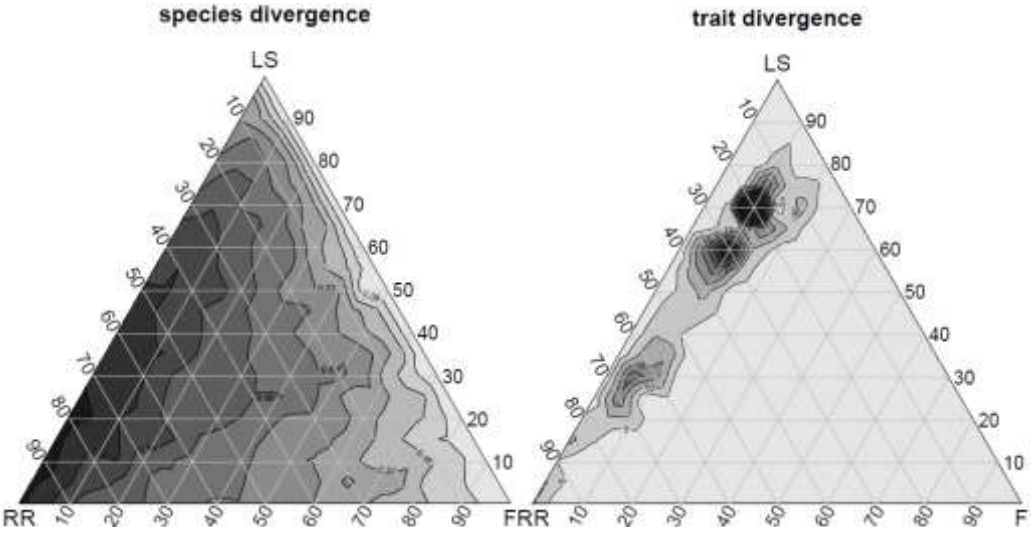


**Fig. B2A.** Response of species (left) and trait (right) convergence to main categories of community assembly processes. Dark colours indicate high divergence, while light colours indicate high convergence. Abbreviations: RR = relative contribution of random removal steps, LS = relative contribution of limiting similarity steps, F = relative contribution of filtering steps.

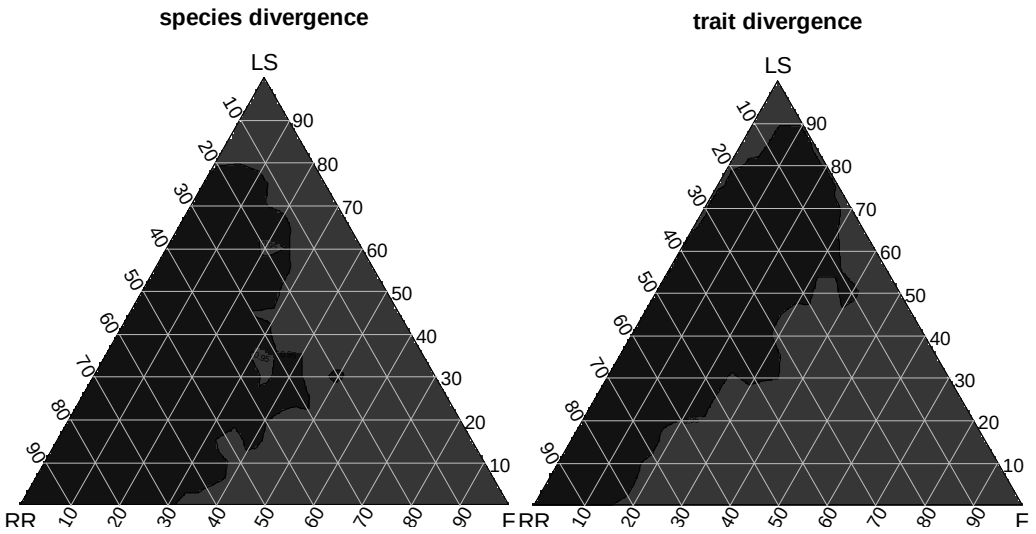


**Fig. B2B.** Species (left) and trait (right) convergence responses to different categories of community assembly processes: differences from random assembly. Colours indicate whether trait convergence values differ significantly from those expected at random (i.e. with 100% random removal steps), with light grey indicating significantly higher convergence, and dark grey indicating no significant differences. Abbreviations: RR = relative contribution of random removal steps, LS = relative contribution of limiting similarity steps, F = relative contribution of filtering steps.

## 9.2 Challenging two widely held assumptions about trait-based community assembly

In trait-based studies on community assembly, practitioners typically assume that trait overdispersion is the result of 'limiting similarity' in traits between species, due to some minimum possible overlap in resource use (resource competition). Trait underexpansion is usually considered to be the result from the 'filtering out' of species not tolerant to the abiotic stress imposed by the environment (Fig. 9.2). Although both these assumptions might sound appealing at first, here I discuss that they are neither unanimously supported by empirical research nor always in line with conceptual or mathematical models from other subfields.

### 9.2.1 'Interspecific competition leads to limiting trait similarity of co-occurring species.'

'Interspecific competition leads to limiting trait similarity of co-occurring species.' This is one of the most widely held assumptions in the field of community assembly, and the idea goes as far back as to Darwin (1859). Assuming that (phylogenetically) related species share similar niches, he stated that 'As species of the same genus have usually, though by no means invariably, some similarity in habits and constitution, and always in structure, the struggle will be more severe between species of the same genus, when they come into contact with each other, than between species of distinct genera.'. This idea regained attention from Hutchinson (1959) and was formalized in models of classical niche theory (MacArthur & Levins 1964). Inspired by this, ecologists have often interpreted trait (Weiher & Keddy 1995a) or phylogenetic (Webb et al. 2002) overdispersion as evidence for competition being the most important process structuring communities. However, both empirical and theoretical studies question the generality of this 'competition-similarity hypothesis' (CSH).

Although the CSH is one of the oldest ecological hypotheses, it has surprisingly rarely been experimentally tested. Among the studies that did so, there are two that found some support (Maherali & Klironomos 2007; Jang et al. 2010). Both these studies used phylogenetic relatedness to quantify similarity between species, assuming that phylogenetically related species are also similar to each other in terms of functional traits. This assumption is by no means necessarily valid (Losos 2009), and consequently the results of these studies should be interpreted with care. The fact that assuming a 'phylogenetic similarity = trait similarity' assumption can lead to wrong interpretations was illustrated in a study that showed that while phylogenetic distance between species is a good predictor of competitive exclusion, trait distance itself is much less so (Violle et al. 2011). Also another experimental study (Johansson & Keddy 1991) testing the CSH for plants with trait data found quite mixed effects. While similarity in species traits related with 'niche properties' indeed led to strong interspecific competition, similarity in species traits related with 'competitive ability' (such as plant height) was more related with the *asymmetry* of competitive outcomes (Johansson & Keddy 1991). Perhaps the largest study testing the CSH was done by Cahill et al. (2008), who investigated with a meta-analysis on single-pair competition experiments whether phylogenetic similarity predicted competition intensity. However, support for the CSH was very weak. Concluding, we thus see that the few studies experimentally testing the CSH do not unanimously show support. There are numerous potential reasons for this: choices on the quantification of 'similarity', the taxonomic breadth of investigated species, abiotic, experimental conditions and many other factors could all bias results. However, there are also theoretical reasons questioning whether one should necessarily expect patterns conform the CSH in the first place.

Contemporary niche theory (Chesson 2000; Chase & Leibold 2003) suggests a more complicated relationship between trait differences and competition intensity between species. According to mathematical models, the coexistence of species is not only driven by *niche differences*. Often, competition can be very asymmetrical (Fig. 9.3, arrow e), which is because the outcome of competition is partially driven by *differences in competitive ability*. This complicates predictions of which species can or cannot potentially co-occur (Chesson 2000). In a recent paper, Mayfield & Levine (2010) explain that species niche differences can be interpreted as trait differences that cause

higher intraspecific than interspecific competition, such as different affinities for certain pH levels (plants) or food plants (animals). Differences in competitive ability, on the other hand, can be thought of as trait differences related to species abilities to acquire a shared type of resources (Mayfield & Levine 2010). An example is plant height, which reflects differences between plant species in capturing light. Taking this idea in mind, we thus see that for the first set of traits, one would expect competition-similarity relationships conform the CSH, while for the latter set of traits no pattern or even the opposite pattern can be expected. A very old study of Johansson and Keddy (1991) in fact already showed empirical support for these predictions made by Mayfield & Levine (2010). Also more recent studies testing whether these different types of traits indeed show different distributions patterns in ecological communities support expectations from Mayfield & Levine (2010) (Anderson et al. 2011; Kunstler et al. 2012).

### 9.2.2 'Species with similar traits are adapted to, and occur in, similar environments, while dissimilar species do not'

Another widely used assumption in studies on trait-based community assembly is that species with similar traits are adapted to similar environmental (abiotic) conditions and therefore also occur in similar environments, while too dissimilar species do not. Based on this idea, observed assemblages of co-occurring species with very similar traits (trait underexpansion patterns) are usually interpreted as being the result of 'abiotic filtering' processes (Weiher & Keddy 1995a). In principle, for the long-term stable occurrence of a species in a certain environment, by definition the species need to be adapted in some way to the given environment. For example, in polar regions one will only find plant species that are able to tolerate severe frost, and this frost tolerance can be seen as a physiological trait shared by all species present in such regions. However, this rather trivial notion is not always very useful: frost tolerance and other life-history traits (growth rate, competitive ability) are very difficult to measure and thus unpractical to use for making ecological predictions. Therefore, in most studies on trait-based community assembly, morphological traits are taken in consideration. It is then assumed that these traits are good proxy's for other traits more closely related to a species niche. For example, in plants, specific leaf area (the area of a fresh leaf divided by its dry weight) is such a trait, which has been shown to be positively related with growth rate and negatively with leaf life expectancy (Westoby 1998). However, variation in one of such 'proxy' trait alone does not always tell us enough about whether a species tolerates certain environmental stress: sometimes there are 'alternative designs' (Marks & Lechowitz 2006) possible that lead to tolerance of the same environmental stress. For example, plants have evolved several very different strategies to cope with fires (Bond & Wilgen 1996) that can be classified as tolerance versus avoidance strategies. Concluding, although it is reasonable to assume that adaptations to a certain abiotic stress should be reflected by traits and that as a result, in stressful environments, species should be similar *in at least some* (difficult to measure life-history) traits to co-occur, they are not necessarily similar in *any* kind of trait. In some cases, it may be very hard to measure the right traits to detect abiotic filtering, unless one is certain about the function of trait under investigation. Therefore, when trait underexpansion patterns are not detected, one should think critically whether the right traits have been measured.

## 9.3 Adding ignored ecological processes to trait-based community assembly theory

Ecological communities are assembled by different processes, such as stress, competition, trophic interactions, facilitation, ecosystem engineering effects and evolutionary history (Fig. 9.3). Therefore, it is worrying that the literature on trait-based community assembly largely ignores other processes than abiotic filtering and limiting similarity (Fig. 9.2). In this section, I will discuss how these 'missing processes' could play a role in community assembly and how their effects are mediated by functional traits.

### 9.3.1 Bottom-up effects

To survive, all organisms need food. For animals, this food consists of other organisms: plants, other animals, or both. Therefore, when bottom-up processes predominate in community assembly and animals are at least to some extent choosy about their food species, community patterns of higher trophic levels can only be better understood when taking community patterns of lower trophic levels into consideration (Fig. 9.3, arrow h) (Belya & Lancaster 1999). Nonetheless, studies investigating this are extremely rare. In an early study on Darwin's finches, it was shown that their community assembly could be explained by the availability of differently sized seeds, with large finch species usually having large beaks and therefore preferring to feed on larger seeds than small finch species (Schluter & Grant 1984). Whether there were also links between within-community seed distribution patterns and finch (beak) size distribution patterns was not tested. **Chapter 4** contains to my knowledge the first and thus far only example where the links of trait distribution patterns of co-occurring prey and consumer communities were investigated. In Hluhluwe-iMfolozi savannah grasslands, variation in grasshopper trait expansion patterns appeared to be best explained by variation in trait distribution patterns from grass communities. For grasses, on the other hand, variation in trait expansion patterns was explained by variation in environmental conditions. Thus, trait distribution patterns in prey communities resulting from certain assembly mechanisms can have cascading effects on higher trophic levels. Interestingly, these moderate, but significant patterns were found for a group of generalist herbivores. For more specialist herbivore species, such as certain beetle groups (Becerra 1997), trait distribution patterns between predator and prey communities might be even much more tightly linked, which would be an interesting hypothesis to test in future studies.

### 9.3.2 Top-down effects

When organisms feed on other organisms, a trivial consequence is that they can also be consumed by other organisms, their 'consumers'. These consumers include predators, parasites, parasitoids and pathogens. In some cases, prey species are unable to live in environments where certain consumer species are present. Such top-down effects of consumer communities on the assembly of prey communities could be mediated by traits (Fig. 9.3, arrow i). One effect that consumers could have on prey community trait distributions, is limit their similarity (Abrams 1983). Consumers, especially invertebrates or microorganisms (which are the majority of species) are often monophagoues or oligophagous (Becerra 1997). When being monophagous, they often have negative, density dependent effects on species (Janzen 1970; Connell 1970), contributing to species diversity (Petermann et al. 2008). Similarly, when being oligophagous and feeding on a small set of similar (e.g. in terms of alkaloids) species, they could limit trait similarity by only allowing one of their preferred prey species to persist in the local environment (Abrams 1983). Generalist, polyphagous consumer species could have opposite effects on trait distributions of co-occurring prey species: they could act as a 'predation filter' excluding prey with certain traits (e.g. obligate sexual reproduction in plants or intermediate body size in herbivores, as suggested in **chapter 5**), causing trait underexpansion (Belya & Lancaster 1999; Pakeman 2011).

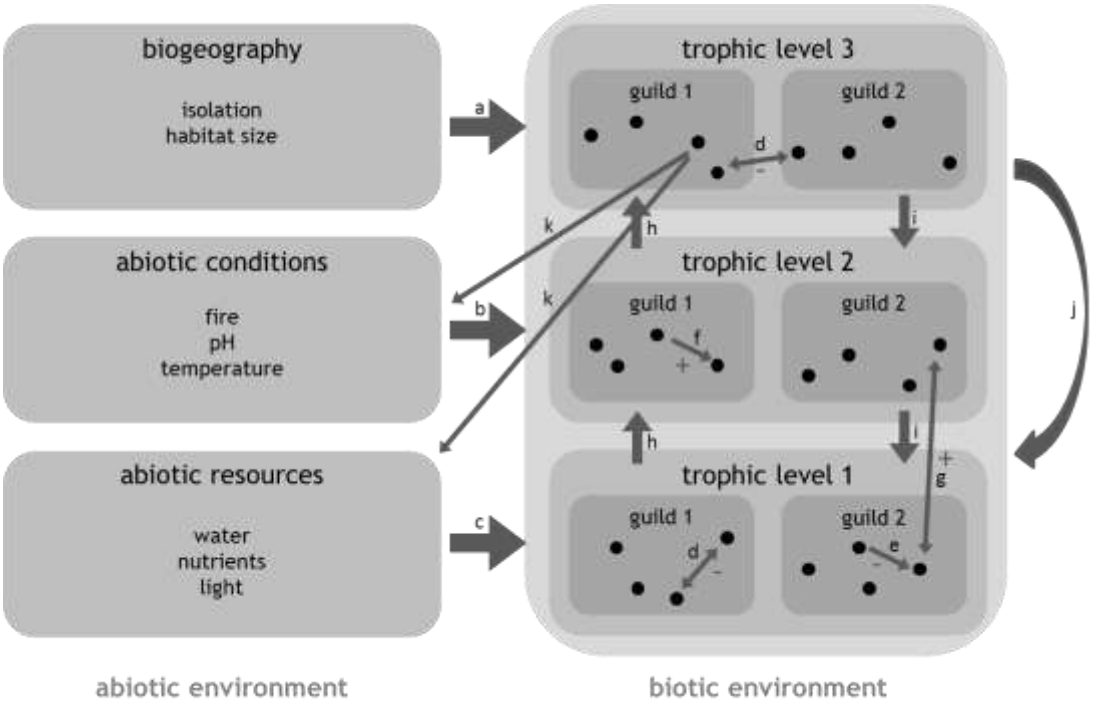


**Figure 9.3.** A conceptual idea of community assembly. Here, community composition is both affected by the availability of abiotic resources (arrow c), abiotic conditions (arrow b), biogeography (arrow a) and the biotic interactions (arrows d-i) and evolutionary history (arrow j). Biotic interactions are include symmetric competitive interactions (arrows d), both within and between guilds, asymmetrical competition between more and less competitive species (arrow e), facilitation (arrow f), mutualisms (arrow g), bottom-up trophic effects (arrows h) and top-down trophic effects (arrows i).

Studies experimentally investigating the effects of consumers on trait distribution patterns of prey communities usually focused on large, mammalian grazer species as the consumers under investigation. These rather polyphagous species often have strong top-down effects on plant communities (Milchunas & Lauenroth 1993; Olff & Ritchie 1998), with often cascading effects on arthropods (**chapter 3**). The top-down effects on plant communities are reflected by trait shifts of dominant species: grazing generally promotes short, annual, horizontally growing species with small leaves and high specific leaf area (Diaz et al. 2001;2007, **chaper 2**). Other effects are more context-dependent: effects of grazing on the digestibility of dominant plant species tend to be negative in unproductive systems and more positive in productive systems (Coley 1988). The few studies that investigated whether grazing also affects trait distribution patterns of plant communities, show more mixed effects. Pakeman (2011) showed that grazing led to trait underexpansion in plant communities, in line with the idea that generalist enemies can act as a 'predation filter' in plant community assembly leading to a set of species with similar traits. In the study of **chapter 2**, I show that in African savannahs, grazers did not have the same effect: both in ungrazed, rhino grazed and heavily grazed grass communities, trait distribution patterns did not differ from random (unpublished results). Possibly, all grass species from this ecosystem are at least to some extent adapted to grazers, so that grazers do not act as a 'predation filter'. Alternatively, the fact that grazers leave some patches ungrazed even in the 'grazed' treatment (Cromsigt & Olff 2008) could have masked effects.

Furthermore, it should be noted that these large herbivores do not only act as 'consumers' of plants, but also as ecosystem engineers (**chapter 7**), and it might be that consumption and ecosystem engineering effects of large herbivores are in some ways opposing each other.
Smaller herbivore or other small consumer species tend to be (but are by no means always) more oligophagous than large ones (e.g. Becerra 1997; Feranec 2007). Therefore, in studies looking at the effects of small (e.g. microbe or arthropod) consumer species on plant trait distribution patterns, one might often expect them to cause limiting trait similarity in at least some cases. In line with this, in rainforests, pathogens have been attributed to cause reduced survival of tree seedlings growing near phylogenetically related species (Webb et al. 2006; Gilbert & Webb 2007; Metz et al. 2010). Furthermore, for plant lineages that have co-evolved with arthropod herbivores, chemical anti-herbivory traits have been found to be overdispersed (Becerra 2007; Kursar et al. 2009). Additionally, herbivory damage of exotic plant species grown together with native species in a common garden experiment has been shown to be higher when the exotic species was highly related with native ones (Hill & Kotanen 2009). However, other evidence opposes the view that 'small' or oligotrophic consumers necessarily drive trait limiting similarity in prey communities. In natural populations, Hill and Kotanen (2009) did not observe the same limiting similarity patterns that were found in a common garden, suggesting that there are factors that oppose the limiting similarity effects of herbivores. Also, in savannah tree communities, traits against arthropod herbivory were underexpanded rather than overdispersed (Loiola et al. 2012). We thus conclude that there is quite mixed evidence that smaller consumers cause limiting trait similarity in prey populations. More experimental research on this topic, involving consumer species that have been proven to be oligophagous, might solve this problem in the future.
An interesting point is that by affecting prey trait distributions, consumers can indirectly also affect trait distributions of other guilds linked with their prey. For example, many grazing exclosure experiments show that grazers usually promote relatively small-sized, thermophile arthropods over other species (**chapter 2,3**). This suggests that large grazers could not only affect plant trait distribution patterns, but possibly also trait distribution patterns of insects. However, in a study where we tested this idea, we did not detect effects of large grazers on arthropod trait distribution patterns (**chapter 2**).

### 9.3.3 Positive species interactions

While studies in community ecology tend to focus on negative species interactions, positive effects are ubiquitous as well (Fig. 9.3, arrow f,g) (Bertness & Callaway 1994). These positive interactions have traditionally largely been ignored in studies of trait-based community assembly, but since recently there is a call for integration of these (Butterfield & Callaway 2012).
One example of positive species interactions is facilitation between species from the same trophic level (Fig. 9.3, arrow f). Facilitation between plants has been shown to be especially important in environments with strong, non-resource based stress (Callaway et al. 2002) or in environments where predation by higher trophic levels is strong (Maestre et al. 2009). There, facilitation could promote the survival of species that are poorly adapted to abiotic stress, reducing effects of abiotic filtering (Butterfield & Callaway 2013). This conceptual idea was empirically demonstrated in Alpine systems, where many plant species cannot persist the severe abiotic conditions without the facilitation by functionally dissimilar cushion forming plant species (Schöb et al. 2012; Spasojevic & Suding 2012). A similar example exists in more arid systems, where nurse plants facilitate other, unrelated plants through the modification of soil and microclimatic conditions, thereby opposing effects of abiotic filtering (Valiente-Banuet & Verdú 2007). These examples show that positive, facilitative species interactions can have surprisingly similar effects on within-community trait distributions as negative species interactions (competition).
Another example of positive species interactions are mutualisms between flower-producing plants and animal pollinators (Fig. 9.3, arrow g). McEwen and Vamosi (2010) proposed two competing hypotheses on the effects that pollination could have on trait distributions patterns of plant communities: in case that interspecific pollen transfer (which is expected to occur most frequently

between species with similar flowers) has strong negative effects on plant reproduction, pollination should lead to overdispersion of flower traits. Alternatively, if plant species with similar flowers affect each other positively due to the attraction of shared pollinator species, flower traits should be underexpanded. The former pattern was observed, supporting the 'interference competition for mutualists' hypothesis (McEwen & Vamosi 2010). This conclusion was later supported in a similar system (Eaton et al. 2012). A question that arises from these studies, is whether there is also a feed-back of flower communities on pollinator trait distribution patterns and whether other mutualistic interactions (e.g. between plants and seed dispersers) also affect trait distribution patterns.

### 9.3.4 Ecosystem-engineering

Ecosystem-engineers are organisms that affect populations of communities of other species by (indirectly) altering the availability of resources or by changing abiotic conditions (Fig. 9.3, arrows k) (Jones et al. 1994). Although almost all species can be considered ecosystem-engineers, the term 'ecosystem engineering' is nonetheless useful for describing the non-trophic interactions by which an organism affects other species positively or negatively, especially when a single species strongly affects a whole set of others (Laland et al. 1999). Broadly, ecosystem-engineering effects van be divided in two types: (1) ecosystem-engineering effects that 'relax' environmental conditions or ecosystem-engineering effects that make environmental conditions more stressful for other species. Examples of the first type include the construction of mounds with fertile, moist soils by termites or the fixing of nitrogen by legume plants, making surrounding soils more fertile. Examples of the latter type include the trampling of ungulates making wet soils more anoxic (Schrama et al. 2013) or dry soils even more dry (Belsky 1986). While the former type is expected to decrease effects of abiotic filtering, the latter is expected to lead to trait underexpansion patterns.

**Chapter 6** provides another example of an ecosystem-engineer that is expected to relax abiotic stress: mound constructing termites. Mound building termites are shown to promote tree communities that are dominated by non-spiny species with relatively large, simple leaves, low wood density and low nutrient contents. This was attributed to the relatively benign conditions (high water and nutrient availability, low fire risk) that one finds on termite mounds, as opposed to abiotic conditions in surrounding soils. Surprisingly, this shift in community composition did not lead to shifts in within-community trait distribution patterns (unpublished results of **chapter 6**). Interestingly, these mound building termites might indirectly even have effects on other trophic levels: large browser species generally prefer to feed on tree species with high leaf nutrient contents which are rare on termite mounds, suggesting that termite mounds are potentially less visited by large browser species (**chapter 6**). **Chapter 7** provides an example of an ecosystem engineer that is expected to increase abiotic stress. When large herbivores move through the savannah, they trample the soil. As a result, soils from grazing lawns appeared to be much more compacted than soils less visited by ungulates, and probably due to this compaction, water infiltration also appeared to be lower in these soils. In a microcosm experiment, it was subsequently shown that the grass species dominating on these dry soils had relatively high growth rates, more horizontal growth, lower root biomass and higher specific root length, possibly as an adaptation to grow in dry areas. Concluding, we thus see that ecosystem-engineering does affect the traits of the species dominating communities, but effects on trait distribution patterns are still not understood well enough.

### 9.3.5 Evolutionary processes

Although non-random trait distribution patterns are usually suggested to be the result of niche-based ecological processes structuring communities, they do not need to be so necessarily. Past evolutionary events could have a significant impact on trait distributions within ecological communities. Although I am not aware of empirical examples, I will explain under which conditions evolutionary events could lead to non-random trait distribution patterns, even in the absence of niche-based ecological processes.

In cases were dispersal is highly limited, such as between islands of archipellos, speciation could lead to phylogenetic underexpansion, since new species are very related to 'parent species', but unable to

migrate to islands dominated by unrelated species (Johnson & Stinchcombe 2007). On the other hand, allopatric speciation could lead to phylogenetic overdispersion when recently diverged species come into secondary contact (Johnson & Stinchcombe 2007). Thus, spatially explicit evolutionary processes alone can already lead to non-random patterns in phylogenetic similarity of co-occurring species (Emerson & Gillespie 2008). If functional traits are phylogenetically conserved, similar non-random patterns could be expected for trait distribution patterns.

## 9.4 Putting all processes together: what do trait distribution patterns tell us avout community assembly?

We have so far seen that all ecological processes can affect trait distribution patterns, and often effects of different ecological processes are very similar. Additionally, the occurrences of different ecological processes are not mutually exclusive: the composition of a community can be the result of e.g. both abiotic filtering, predation *and* competition processes. This has led several researchers to suggest that it is very hard (Kraft et al. 2007), if not impossible (Mayfield & Levine 2010), to conclude anything about community assembly processes based on trait distribution patterns. Although I agree that with the notion that it is *hard* to link patterns to processes in trait-based community assembly, I am optimistic and think it is *not impossible* to do so, as I will explain here.

A very large number of processes are responsible for shaping communities, involving many historical contingencies and specific cases (Lawton 1999). However, for understanding community assembly, it can help to temporally 'lump' different processes into three main categories (Fig. 9.4), which are defined by their main effects on trait distribution patterns (**chapter 8**). The categories I use are firstly: neutral or stochastic processes, which are independent of traits. Secondly, there are filtering processes (see Box 1), which are the processes that tend to decrease trait space in communities (e.g. the filtering out of stress-intolerant species, competitively inferior species or predation intolerant species). Note that this 'filtering' thus has a broader definition than the classic use of 'abiotic filtering' or 'habitat filtering', see Box 1. Thirdly, I identify limiting similarity processes, which are the processes that limit the similarity of co-occurring species (e.g. limiting similarity of shared resources or shared predator species) (**chapter 8**). After lumping ecological processes into these three main categories, we thus see that two of the categories have rather, *but not completely*, opposite effects on trait distributions: filtering decreases trait range (or multidimensional trait space) and thus causes trait underexpansion, while within this trait space, limiting similarity processes tend to decrease the similarity of species, creating trait overdispersion (**chapter 4, 8**). If filtering processes and limiting similarity processes would have exactly opposite effects on trait distribution patterns in communities, trait data would often be insufficient in linking patterns and processes in community. When some measure of within-community trait distribution would not differ from random, this could both mean that stochastic processes have shaped the community, or, alternatively, a combination of limiting similarity and habitat filtering processes. However, if limiting similarity and environmental filtering processes affect slightly different aspects of trait distribution patterns, then different scenarios of community assembly are more likely to cause unique, different patterns in trait distributions (**chapter 8**). In line with this reasoning, several studies used slightly different measures for within-community trait distributions to detect both filtering and limiting similarity patterns in community assembly, both with univariate (e.g. Kraft et al. 2008; Cornwell & Ackerly 2009; Paine et al. 2011, Bernard-Verdier et al. 2012; **chapter 4**) and multivariate (e.g. Villéger et al. 2008; **chapter 8**) approaches. These studies have shown that trait underexpansion and overdispersion patterns are not mutually exclusive (e.g. Cornwell & Ackerly 2009, Bernard-Verdier et al. 2012; **chapter 4**).

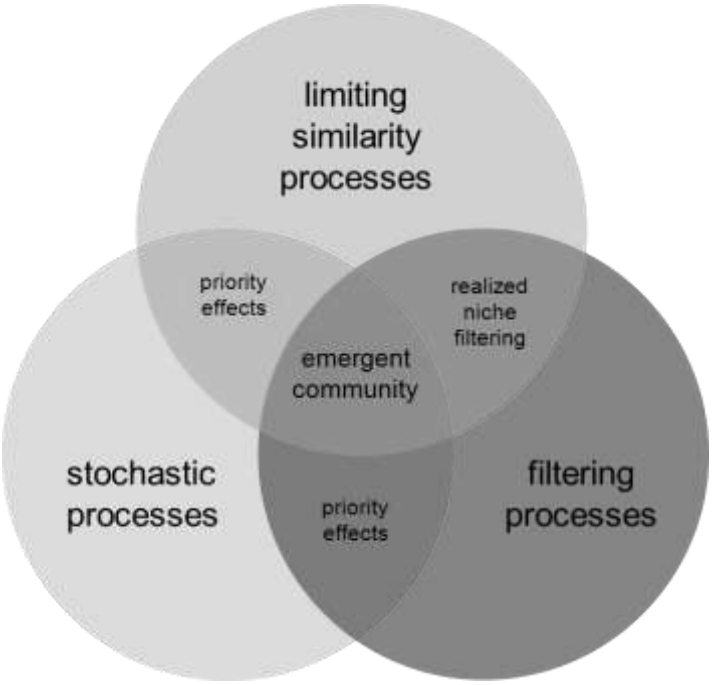


**Figure 9.4.** Community assembly processes can be categorized into three main types shown in this Venn-diagram: (1) 'stochastic processes', in which the presence and abundance of a species in a community is not determined by its (measured) traits, (2) filtering processes, which are niche-based processes filtering out species with unfavourable traits, thereby causing trait underexpansion and (3) limiting similarity processes, in which too similar species exclude each other, causing trait overdispersion. In most cases, communities are structured by more than one of those processes, and together, these processes lead to an 'emergent community'. The interaction of neutral- and niche based processes are often seen as 'priority effects' (Shulman et al. 1983; Berlya & Lancaster 1999), while the abiotic and biotic environment of a species determine a species 'realized niche' via filtering and limiting similarity processes (Weiher et al. 2011; **chapter 4**).

Simultaneously investigating different measures of trait distribution patterns may thus help us to investigate what the relative contributions of different main categories (stochastic, filtering or limiting similarity) of processes to community assembly are. However, traditional null model approaches are usually not suitable for solving these types of questions: they do or do not reject a null hypothesis, and are therefore only suitable for detecting or not detecting the *presence* of certain processes, but not for estimating their *relative contribution* to community assembly. In **chapter 8**, a new modeling approach is developed and used in combination with Bayesian inference to estimate the relative proportion of stochastic, environmental filtering and limiting similarity processes in community assembly. This approach was then applied to tree communities positioned at different locations over fire and rainfall gradients in a South-African savannah, which led to two main conclusions: (1) savannah tree community assembly was mostly regulated by stochastic processes and less so by filtering and limiting similarity and (2) in areas with low fire frequency and high rainfall, limiting similarity processes were relatively more important in structuring tree communities. This study thus demonstrates that within-community trait distribution patterns can successfully be linked with community assembly processes. However, we should keep one thing in mind: as noted before, I opportunistically termed all different processes constraining trait space of co-occurring species

'filtering' processes, all different processes limiting trait similarity within this range 'limiting similarity' processes, while all 'real' stochastic processes, but also niche-based processes that are not mediated by the species traits measured in a certain study are captured under the name 'stochastic processes'. So although the study of **chapter 8** did lead to some conclusions about the relative importance of different processes in community assembly, these conclusions are rather 'coarse': both the stochastic, filtering and limiting similarity processes can be further subdivided into different 'subprocesses'. It is still the question how important all these 'subprocesses', such as limiting similarity through competition, limiting similarity through predation, etc., are in assembling communities. I think it is unlikely that refinements in modeling and observational studies alone are going to bridge that gap. Therefore, I agree with others (e.g. Götzenberger et al. 2012; HilleRisLambers et al. 2012) that in the future, observational studies on trait-based community assembly need to be much more complemented with experimental studies.

## 9.5 Human impacts on biodiversity and trait-based community assembly

Thus far, I outlined what we can infer from within-community trait distributions on community assembly, mostly referring to studies investigating relatively 'natural' or 'pristine' communities. I reasoned that by gaining insight in the processes that shape these 'healthy' communities, as a next step, one can learn to understand how anthropogenic disturbances affect community assembly. As a result, we might be able to learn how this results in 'unhealthy' degraded systems (Fig. 9.5). These insights can then be used to make conservation strategies aimed to restore 'natural' ecological processes in degraded systems.

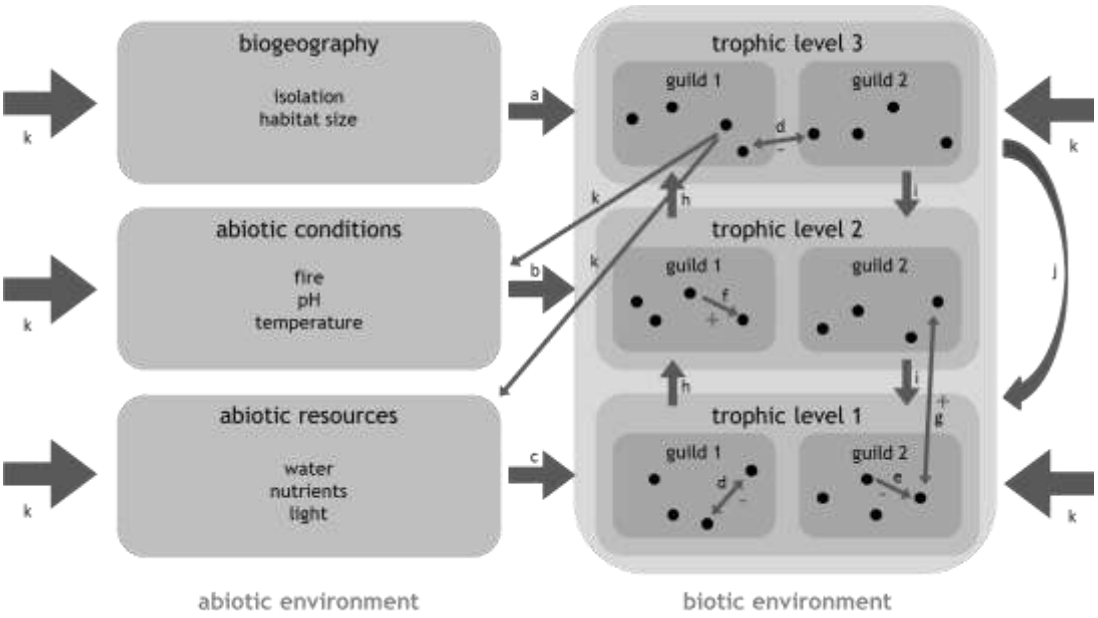


**Figure 9.5.** A conceptual idea of community assembly. Here, besides the 'natural processes' (arrows a-j, see caption Fig. 9.2), also human effects on community assembly are shown (arrows k).

### 9.5.1 Linking trait-based community assembly theory with human threats on biodiversity

In the relatively short time period of a few centuries or millennia, human populations have increased tremendously and humans have highly altered their natural environment. Nowadays, there are hardly any places on earth that are not under direct influence of humans. Many of these influences, such as overgrazing, fires or human-caused droughts, can be seen as 'disturbances' or human-caused stress conditions. Due to the relatively recent expansion of human influences on earth, not all species might be adapted to these. This does not only simply cause species loss, but, assuming that species need specific traits to cope with human-caused disturbances or stress, might also cause shifts in within-community trait expansion patterns. This phenomenom was demonstrated by Pakeman (2011), who found disturbances such as livestock grazing and mowing causing trait underexpansion in Scottish plant communities. Human disturbances can also cause trait underexpansion in a more direct way: the selective logging in a Chinese tropical rain forest has been found to cause trait underexpansion, possibly because tree species with specific traits related with high wood quality are preferably logged (Ding et al. 2012). Human disturbances can thus act as additional ‘filters’ in community assembly, causing trait underexpansion. However, this is not always the case. In herbaceous communities in Costa Rica, trait underexpansion patterns were found for growth form in deforested sites, while underexpansion patters for fruit types were stronger in forests, possibly due to a higher variety of fruit dispersers there (Mayfield et al. 2006). This emphasizes the fact that traits with different functions can respond differently to environmental conditions.

While human influences on natural systems can sometimes be seen as ‘stressful’ disturbances, there are also human influences that cause more 'benign' environmental conditions. For example, it is increasingly recognized that humans are causing global warming (IPCC, 2007, Climate Change Synthesis Report), and this can make environmental conditions in the Arctic less severe. In a monitoring study on stream invertebrate communities near a retreating glacier, within-community trait distribution patterns shifted from trait underexpansion towards more 'random' trait distribution patterns as temperatures rose (Brown & Milner 2012). While 'less stressful' should be no means necessarily be seen as 'preferred', this study demonstrates that human influences on communities can not only decrease, but also increase within-community trait variation patterns. Furthermore, it is important to note that there are many other influences of humans on ecosystems, such as fertilization, pollution, hunting and fishing, which have been shown to highly alter biodiversity, but that have thus far never been investigated in studies on trait-based community assembly.

### 9.5.2 Linking trait-based community assembly theory with biodiversity conservation

In previous sections, I demonstrated that community assembly can be seen as a combination of niche-based and neutral processes shaping communities. These niche-based processes, which can be divided in filtering and limiting similarity processes, impose some 'rules' on community assembly: in certain environments, for example in a desert, species with certain traits, such as thin-leaved plants, cannot survive, leading to a dominance of succulent plant species. Furthermore, biotic interactions can limit similarity of co-occurring species. However, due to 'stochasticity' or neutral processes, it is often almost impossible to predict which *exact set of species* will grow in a certain location (Box 2). The fact that communities are usually structured by both niche-based and neutral processes has some major implications for conservation.

A first implication is that the identification of niche-based 'assembly rules' in natural communities and the shift in 'assembly rules' in human-altered environments can help us thinking of solutions to restore natural community assembly. For example, in disturbed Scottish plant communities, that had a higher tendency for trait underexpansion patterns than more pristine systems (Pakeman 2011), nature restoration could be implemented to make environmental conditions more suitable for species with traits that are absent in disturbed situations. Such ‘function-based nature management’ should then lead to communities containing species with a wider variety of traits. However, these 'restored'

communities might still lack certain ‘target’ species that one wants to protect, even if environmental conditions are suitable.
This is due to stochasticity or neutral events: it is often impossible to exactly predict which species will return to areas with restored abiotic conditions, even if one knows about the traits or 'niches' of target species one wants to protect. 'Assembly rules', following from niche-based processes in community assembly, are relatively adequate in predicting which types of *traits* can occur in certain environments, but not in exactly predicting which *species* will occur in certain environments (Fukami et al. 2005; Weiher et al. 2011; Helsen et al. 2012, Box 2). The question is what conservation managers should do with this information. One solution is just accepting that nature management, even if carried out well, will not always lead to 'target' communities. Although this may sound harsh, one could defend such a perspective by arguing that ‘ecosystem services’ or ‘ecosystem functions’ correlate more strongly with *functional* diversity than with *species* diversity (Diaz & Cabido 2001; Hooper et al. 2005). Prioritizing the conservation of ‘traits’ or ‘functions’, rather than species (‘function-based nature management’), should thus be sufficient for the conservation of ecosystem services or functions. However, one could also argue that such a view ignores the ‘intrinsic value’ of species that most people highly appreciate. Indeed, although the panda (*Ailuropoda melanoleuca*) might functionally play a minor role in its ecosystem, many people are willing to pay large sums of money to finance its conservation. Similarly, when two plant species are functionally (i.e. in terms of traits) very similar, but one is common and the other extremely rare, we often want to protect the rare species (‘species-based nature management’), despite its functional redundancy.
The question is: what to do when, despite suitable (a)biotic conditions in a certain environment, such a rare species is, due to ‘neutral’ processes, nevertheless absent in a nature area, or vulnerable to local extinctions? The solutions one can find by focusing on the nature of stochasticity. One process that is often considered as an important cause of 'neutrality' in community assembly, is 'ecological' (Hubbell 2001) or 'demographic' (Hanski 1998) stochasticity. Due to chance effects, when population sizes are small, species can get locally extinct, despite suitable environmental conditions (MacArthur and Wilson 1967; Hanski 1998; Hubbell 2001). Enlarging nature reserves reduces the chance of such 'stochastic extinctions'. Another process that is largely unpredictable, is dispersion (e.g. Hubbell 2001). Often, species are absent in suitable habitat due to the absence of nearby populations to migrate from (Bakker et al. 1996; Hanski 1998). In such cases, species reintroductions might be fruitful in creating more viable metapopulations, as well as in creating local communities consisting of many preferred, rare 'target species'. Of course, when applying such ‘species-based nature management’, one should be willing to ‘garden’ in nature, thereby taking away more natural ‘chance effects’ of community assembly.
Concluding, I suggest that three important messages for conservation managers can be drawn from community assembly theory: firstly, in some cases, (function-based) nature restoration can restore natural community assembly processes. Secondly, although these restored community assembly processes do not necessarily lead to the return of target species in local communities, large nature reserves with much suitable habitat increase the chance of observing such target species in at least some locations. Thirdly, when target species are absent in a nature reserve, despite a large area with suitable environmental conditions, we can help these target species by reintroducing them (species-based nature management).

## 9.6 Concluding remarks

There is a large and growing body of literature trying to link within-community trait distributions patterns to community assembly processes. However, these trait-based studies on community assembly often still make oversimplified assumptions. Furthermore, trophic interactions, positive species interactions and ecosystem-engineering effects have been often neglected, while within-community trait distribution patterns have been often linked with certain processes without critical discussions on other processes that could have caused similar patterns.
In this thesis, I demonstrated the importance of trophic interactions and ecosystem engineering effects in community assembly, and I showed that these interactions are often mediated by species traits.

These studies complement a slowly growing body of literature on the effects of positive species interactions on within-community trait distribution patterns. Furthermore, I showed how we can use insights from these studies to quantitatively infer the importance of different community assembly processes in nature with models. However, even though I see this approach as a useful step forward in understanding how communities are assembled and in understanding how community assembly might differ within and between ecosystems, I acknowledge that there are also limitations in the insights it can give. For example, when limiting similarity has been found to have a high impact on assembling communities, we still do not whether competition (limiting similarity for shared resources) or trophic interactions (limiting similarity for shared predators or pathogens) have caused this pattern. Therefore I think that in the future, the mostly observational studies on trait-based community assembly should be much more complemented with experimental studies. This should ultimately lead to a better understanding of community assembly and to more effective nature conservation strategies.

# Supplements

## S2: Chapter 2

### S2.1: Extra information about sites.

The exclosures were spread throughout the rainfall gradient in HiP (Fig. S2A), with the most southwestern plot receiving less than 500 mm rainfall per year (long term average) and the wettest site receiving over 700 mm rainfall per year. The rainfall data were obtained from KZN wildlife (KZN wildlife, unpublished data).

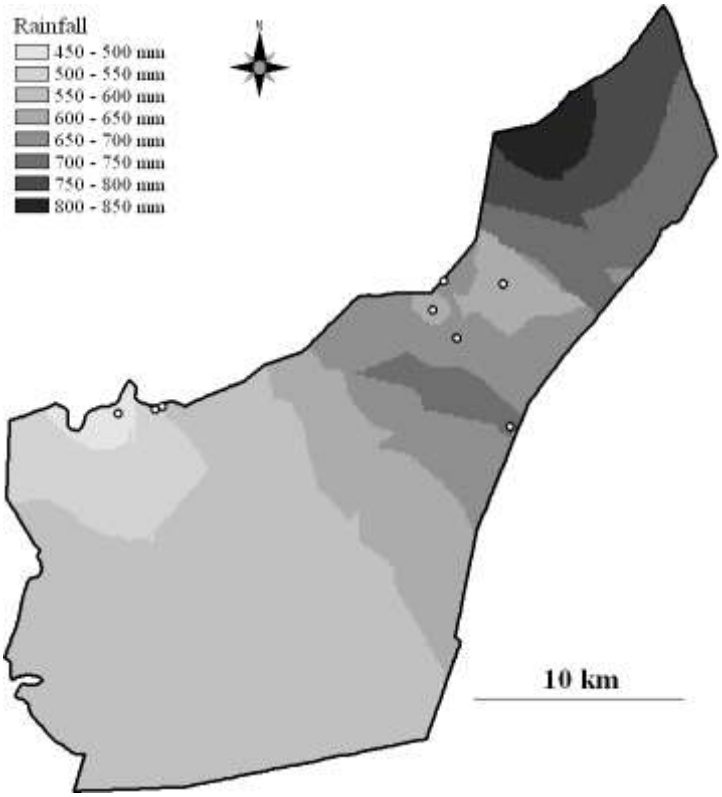


**Figure S2A.** Overview of all the sites visited during this study. The colours of the map show the rainfall gradient, with the driest plot (Thobothi) receiving on average less than 500 mm rainfall per year, while the wettest plot (Le Dube) receives on average over 700 mm rainfall per year.

### S2.2: Extra information about observed grass species

During this study, 24 grass species were observed: 16 bunch grass species and 8 lawn grass species. At each site where the species were observed, traits (LA, SLA, leaf N content and canopy height) were measured, if possible within the control plot. With these trait data, we could calculate Species average Trait Values (STVs): see Table S1A. In general, lawn grasses were shorter and had smaller leaves than lawn grasses, while leaf N content was higher. *Panicum maximum*, a common bunch grass associated with hare fence plots, deviated from this trend: contrary to most bunch grass species, it has high N contents in its leaves (Table S2A).

**Table S2A.** List of all grass species found during this study. Grass species were sorted in two types: vertically growing, mostly sexually reproducing bunch grass species and horizontally growing, often clonally reproducing lawn grass species. Column c, r and h indicate in how many (out of 8) control, rhino fence and hare fence plots the species was observed. The STVs are given in the LA, SLA, N and height columns. Habitat indicator species are underlined in either the c and r or in the h column, depending on whether they were associated with high intensity grazing or low intensity grazing plots respectively.

| Species name | type | c | r | h | LA | SLA | N | height |
|---|---|---|---|---|---|---|---|---|
| *Bothriochloa insculpta* | bunch | 5 | 3 | 2 | 5.43 | 211.45 | 2.06 | 56.50 |
| *Brachiaria brizantha* | bunch | 0 | 0 | 1 | 20.85 | 170.00 | 1.54 | 67.40 |
| *Chloris gayana* | bunch | 2 | 2 | 0 | 6.20 | 178.72 | 1.58 | 67.00 |
| *Cymbopogon excavatus* | bunch | 2 | 2 | 1 | 16.94 | 192.09 | 1.73 | 83.80 |
| *Digitaria eriantha* | bunch | 1 | 2 | 4 | 13.65 | 162.79 | 1.49 | 63.68 |
| *Eragrostis curvulata* | bunch | 3 | 3 | 1 | 8.44 | 160.43 | 1.52 | 42.59 |
| *Eragrostis superba* | bunch | 3 | 4 | 2 | 6.71 | 213.44 | 2.29 | 28.13 |
| *Heteropogon contortus* | bunch | 1 | 2 | 0 | 5.48 | 219.13 | 1.59 | 35.88 |
| *Hyparrhenia filipendula* | bunch | 0 | 0 | 1 | 4.46 | 194.85 | 1.54 | 69.80 |
| *Hyparrhenia hirta* | bunch | 1 | 1 | 0 | 13.38 | 181.85 | 1.53 | 81.00 |
| *Panicum deustum* | bunch | 2 | 2 | 2 | 19.14 | 220.56 | 2.23 | 71.52 |
| *Panicum maximum* | bunch | 8 | 7 | *8* | 13.48 | 301.78 | 2.72 | 51.48 |
| *Setaria sphacelata* | bunch | 1 | 2 | 0 | 11.22 | 205.49 | 1.80 | 65.63 |
| *Sporobolus pyramidalis* | bunch | 5 | 3 | 2 | 12.84 | 150.18 | 1.40 | 72.40 |
| *Themeda trianda* | bunch | 4 | 5 | 6 | 7.23 | 227.03 | 1.79 | 63.68 |
| *Tristachya leucothrix* | bunch | 1 | 1 | 1 | 9.80 | 162.82 | 1.16 | 42.40 |
| **average value (± SEM)** | | | | | 10.48 (1.3) | 220.3 (11) | 1.93 (0.1) | 59.54 (4.2) |
| **average value without *Panicum* (± SEM)** | | | | | 9.70 (1.3) | 196.2 (10) | 1.72 (0.1) | 57.87 (4.7) |
| *Aristidia congesta* | lawn | 2 | 1 | 0 | 1.26 | 187.69 | 2.15 | 14.24 |
| *Dactyloctenium australe* | lawn | 1 | 1 | 0 | 2.38 | 361.76 | 2.57 | 17.80 |
| *Digitaria argyrograpta* | lawn | 0 | 0 | 1 | 3.70 | 317.26 | 3.00 | 6.40 |
| *Digitaria longiflora* | lawn | 5 | 5 | 5 | 1.72 | 272.55 | 2.25 | 19.30 |
| *Panicum coloratum* | lawn | 4 | 2 | 3 | 6.73 | 213.40 | 2.42 | 17.75 |
| *Sporobolus nitens* | lawn | *4* | *4* | 0 | 3.05 | 196.81 | 2.80 | 14.83 |
| *Tragus berteronianus* | lawn | 0 | 0 | 0 | 0.87 | 214.09 | 3.43 | 7.85 |
| *Urochloa mosambicensis* | lawn | 5 | 4 | 2 | 7.77 | 279.98 | 2.00 | 33.62 |
| **average value (± SEM)** | | | | | 3.44 (0.9) | 255.4 (22) | 2.58 (0.2) | 16.47 (3.0) |

# S4: Chapter 4

## S4.1: Study area

Our fieldwork was carried out in Hluhluwe-iMfolozi Park, an 89,665 ha nature reserve in Kwazulu-Natal, South Africa (Fig. S4A). HiP is a highly heterogeneous game reserve (Whateley and Porter 1983, Owen-Smith 2004), with the heterogeneity resulting from gradients in altitude (ranging from 50 till 500 m), rainfall (Fig. S4B), fire frequency (Fig. S4C), hydrology and soil heterogeneity.

Throughout the park, 50 grassland plots of 10 x 10 meters with a woody coverage below 15% were chosen that together covered the whole rainfall and fire frequency gradients (Fig. S4B,S4C). All plots were at least 25 m apart from each other, which is far enough to be sure that individual grasses and grasshoppers from different plots do not interact with each other and that all the studied plots from our highly heterogeneous study area can be considered as independent replicates. Many plots were much further apart than this, up to 5.32 km to the nearest plot. The average distance between plots was 918 m. No plots are located in the most southern part of the park, which is managed as a wilderness area and therefore is not accessible for research.

Plots were chosen in such a way that they covered the whole rainfall gradient in the park (Fig. S4B). Rainfall ranged from approximately 450 mm per year in the southwest of the park till approximately 850 mm per year in the north. Furthermore, plots were chosen in such a way that they more or less covered the whole fire frequency gradient in the park (Fig. S4C). Between 1956 and 2004, the most frequently burnt places in HiP burnt 28 times, while other places (mainly very moist places near rivers

or places near lodges) did not burn. Across the natural grassland locations in HiP, the range in fire frequency was a bit narrower. The plot with the lowest fire frequency in our study burnt 4 times between 1956 and 2004, while the most frequently burnt plot burnt 24 times.

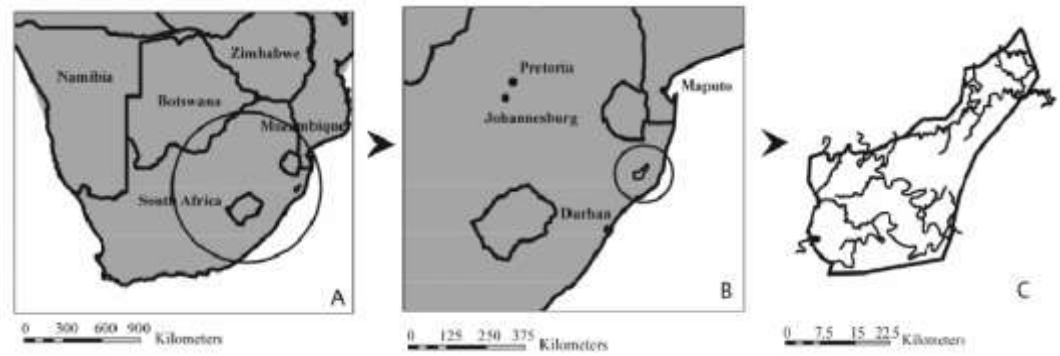


**Figure S4A.** Location of Hluhluwe-iMfolozi Park (HiP), South Africa. a. Southern Africa. b. A more detailed map of the eastern part of South-Africa that is encircled in a. c. a map of HiP with its main rivers, which is encircled in b. Figure copied from Cromsigt, J.P.G.M. (2006) Large herbivores in Space (pp 13) (Doctoral thesis, University of Groningen).

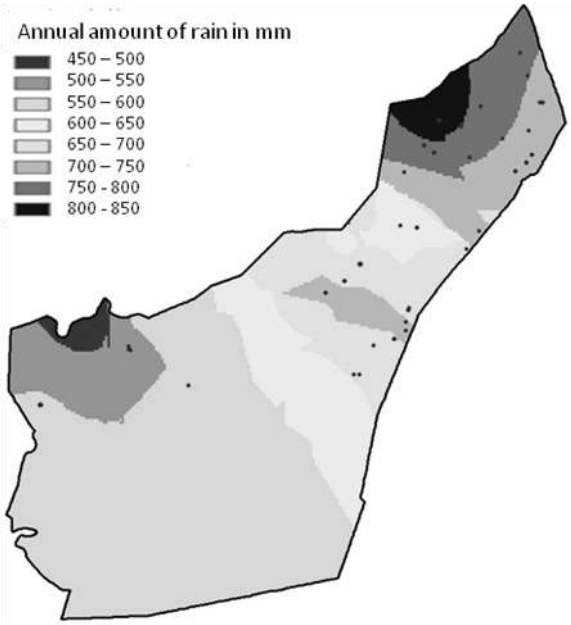


**Figure S4B.** Mean annual amount of rainfall in HiP over the years 2001-2007. The green dots represent the plots were fieldwork was carried out.

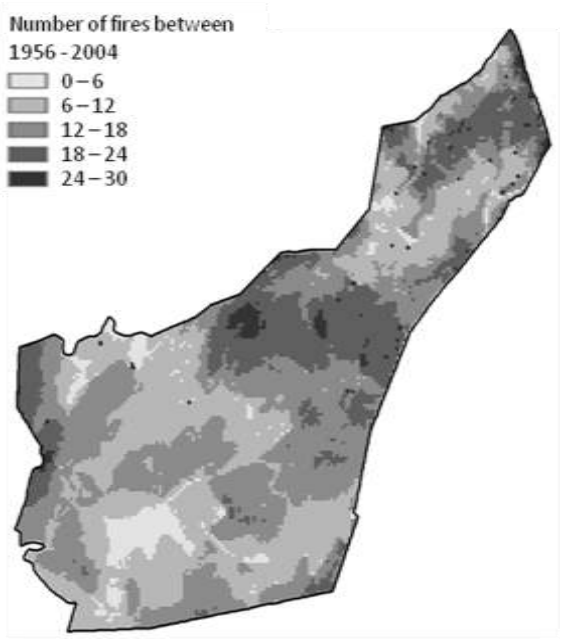


**Figure S4C.** Annual amount of fires in HiP over the years 1956-2004. The green dots represent the plots were fieldwork was carried out.

## S4.2: The development of null models

Different community assembly mechanisms are expected to have different and sometimes opposite effects on the similarity of traits of co-occurring species (Cavender-Bares et al. 2004; Kraft & Ackerly 2010). The two main processes considered important in the assembly of traits are the filtering of abiotic conditions, constraining the trait range of co-occurring species, and competition for resources, causing a large evenness of distances between adjacent trait values of co-occurring species (Cornwell and Ackerly 2009). To study the effects of both processes on community assembly, we created two different null models: a simple null model (Null Model 1) to investigate the filtering of abiotic conditions on trait assembly and a two-step model (Null Model 2) to study the effect of competition on trait assembly.

*Null Model 1*

According to classic theories in trait-based community assembly, the filtering of abiotic conditions is expected to only allow those species in a community that share certain traits (Weiher & Keddy 1995a; Weiher et al. 1998, Cornwell & Ackerly 2009). For example, in arid environments with most of the rain falling in winter, the vegetation mainly consists of succulent plant species flowering in winter (Cowling et al. 1999). The expected result is a community with species having trait (in this case, SLA and first flowering date) values that fall within the constrained range that the environment allows (Fig. S4D) However, we argue that the filtering of abiotic conditions is not the only process that constrains trait ranges in natural communities. Rather, each filter that corresponds on the fundamental niche on species should only allow species in local communities that fall within a certain trait range. For plants, fundamental niches of species are indeed mostly determined by abiotic factors, such as stress conditions (e.g. low pH, frost, fire, salt) or limitations in resources (e.g. water, nitrogen, phosphorous, potassium). However, for animals, the resources that correspond on their fundamental niche are mostly biotic, such as grasses for grazers, tree leaves for browsers or animals for predators.

Hence, if one were to draw a random set of species from the species pool (e.g. all the species found during the study or all the species occurring in the region), the scaled range of trait values of the randomized community (Randomized Trait Range) should be higher than the scaled range of trait

values from an observed community (Observed Trait Range) with the same species richness, that has been formed by strong fundamental niche filtering or the classic abiotic filtering (compare Fig. S4D with S4E). To test this, we performed 10.000 random draws from the species pool, that is, all the species sampled in this study, without replacement, at each observed species richness. The chance of drawing a certain species was proportional to its relative abundance in the species pool: species that occurred in many plots also had a proportionally higher chance of ending up in a randomized community. In this way we prevented false positives caused by an over-representation of rare species with extreme trait values in the randomized communities. The average Randomized Trait Range was then compared with the Observed trait range from communities with the same species richness, and the Residual Trait Ranges (Residual Trait Range = Observed Trait Range – Randomized Trait Range) was calculated for each 10 x 10 m plot. Residual Trait Range was calculated in R-2.9.2 with the help of the R boot package (Canty & Ripley 2009). For the input files, see Supplement 3 and 4 and for the scripts for calculating Residual Trait Range, see Supplement 1.

*Null model 2*

Competition for resources and the resulting limiting similarity (Hutchinson 1959; Macarthur & Levins 1967) is expected to result in a set of co-occurring species with relatively evenly spaced distances between traits (Macarthur & Levins 1967, Pacala & Tilman 1994, Cornwell & Ackerly 2009). The assumption here is that if one chooses traits important in acquiring limited resources, trait differences reflect differences in niches and species with too similar niches are expected to exclude each other. This should result in a community with species that all have a certain minimal distance in traits values to adjacent species, which causes a high evenness in the distances between adjacent traits. As a result, if one were to draw some random traits from a species pool and one compares the trait evenness of such randomized communities (Randomized Trait Evenness) with the trait evenness of observed communities (Observed Trait Evenness), that are formed by strong interspecific competition, one would expect trait evenness to be higher in the observed communities (Fig. S4D and S4F). However, the effects of abiotic filtering and the consequent constrained trait range could also have its impact on the trait evenness in a community. Therefore, to test whether competition may play a role in the assembly of traits in communities, we created a two-step null model. Firstly, the unscaled trait range for each observed plot was calculated. Then, we selected the highest and lowest observed trait values of the given plot and S - 2 (in which S is the observed species richness) randomly chosen traits without replacement that fell within the Observed Trait Range. Again, the chance of picking a certain species for a randomized community was proportional to the species relative abundance. This way, we created randomized communities with the same trait range and species richness as in the observed communities, thereby correcting for the effects of abiotic filtering. For each observed community, we performed 10.000 randomizations. For both the observed and randomized communities, we calculated the (average) trait evenness, which was defined as:

$$\mathrm{TE} = \frac{1}{\mathrm{sdT} + 1},$$

in which sdT is the standard deviation of distances between adjacent trait values and TE is Trait Evenness. Note that a community with perfectly spaced adjacent species trait values has an sdT of 0 and therefore a Trait Evenness of 1, while a community with an extremely high unevenness in adjacent trait values has a very large sdT and a Trait Evenness close to 0. Finally, we calculated the Residual Trait Evenness, defined as Observed Trait Evenness - Random Trait Evenness. Residual Trait Evenness was calculated in R-2.9.2 with the R boot package (Canty & Ripley 2009). For the script to calculate Residual Trait Evenness, see Supplement 2 and see Supplement 3 and 4 for the input files.

*Size of communities and species pool*

Note that choices on the spatial scale of the community and the species pool are non-trivial, since different assembly mechanisms are tended to predominate at different spatial scales. Earlier studies showed that competitive processes acting on community assembly are mainly important at the local

scale, while the filtering of abiotic conditions becomes more important at the larger scale (e.g. Silvertown et al. 2006). This was also predicted by Keddy & Weiher (1999), since it is unlikely that for example relatively small, sessile organisms such as grasses interact with other grasses that are over 100 meter apart, while the abiotic conditions of two sites 100 meter apart may still be very similar, suggesting a relatively large role for environmental filtering at larger spatial scales. In this study, we chose to define all the sampled species as the species pool, assuming that we found the majority of species occurring in our study area and that those species were not too much constrained by dispersal. Furthermore, we sampled in plots measuring 10 x 10 meters and defined communities as such, since these plots should be small enough to detect competitive interactions, while big enough to find enough species that are needed to allow the detection of statistically significant patterns in trait assembly.

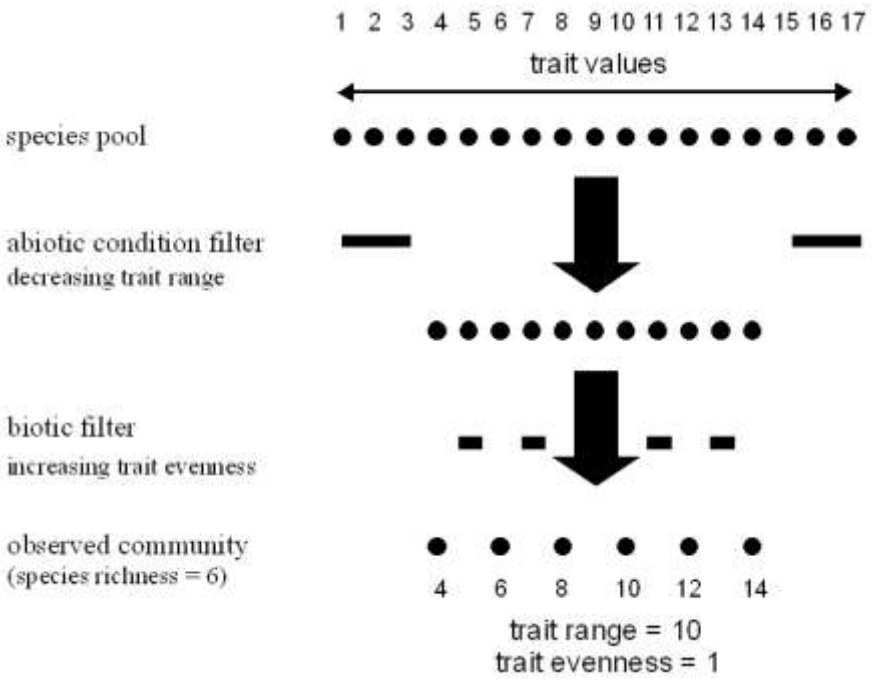


**Figure S4D.** Conceptual idea for the assembly of communities sensu (Diaz et al. 1998) and (Weiher et al. 1998). Firstly, species go through an abiotic condition filter, that only allows species with traits falling within a certain range. Of the species that are allowed by the abiotic condition filter, some will not assemble in the final local community, due to competitive exclusion. Competition and the consequent resource partitioning between species results in a relatively large evenness of trait distances within this range.

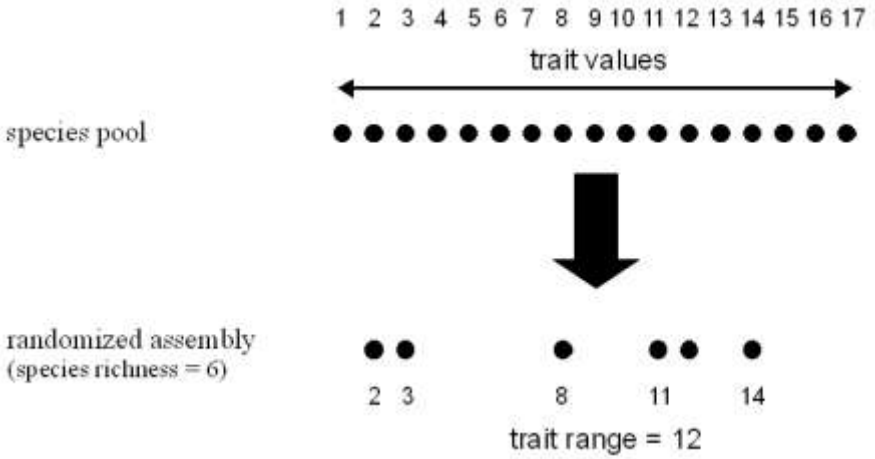


**Figure S4E.** Conceptual overview of Null Model 1. If in an observed community six species were found, the Observed Trait Range of that community was compared with the trait range of six randomly assembled species from the species pool (Randomized Trait Range). If environmental filtering is strong, the Observed Trait Range is expected to be lower than the Randomized Trait Range (compare figure B1 with B2), with the result that Residual Trait Range (= Observed Trait Range – Randomized Trait Range) is negative.

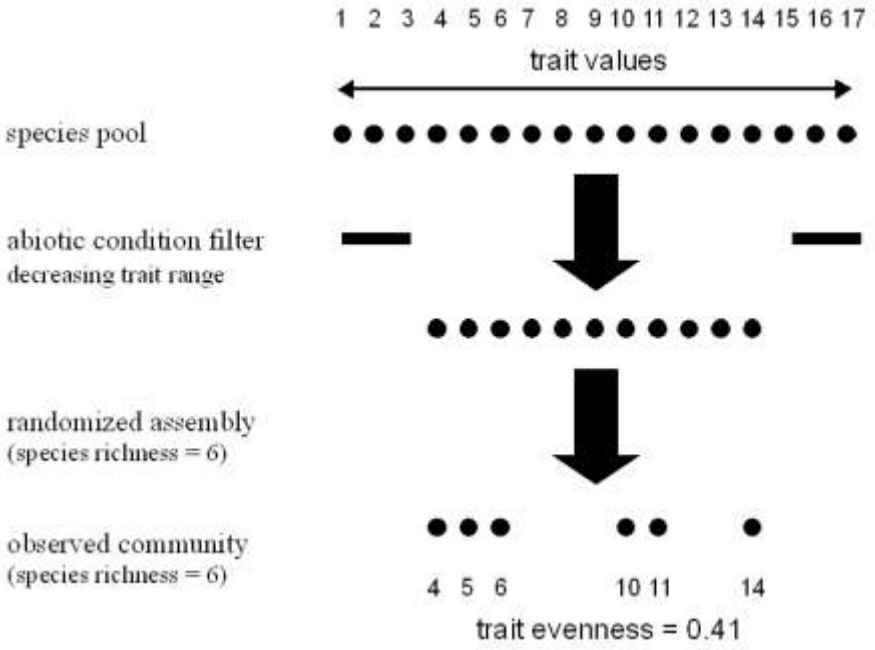


**Figure S4F.** Conceptual overview of Null Model 2. If in an observed community six species were found, the Observed Trait Evenness of that community was compared with the trait evenness of six species, of which one species was the species with the highest observed trait value in the given community, one species was the species with the lowest trait value in the given community and the remaining 6 – 2 = 4 species were randomly drawn from the species in the species pool that had trait values falling within the Observed Trait Range. If competition and limiting similarity are strong, the

Observed Trait Evenness is expected to be higher than the Randomized Trait Evenness (compare Fig. S4D and S4E), with the result that Residual Trait Evenness (= Observed Trait Evenness – Randomized Trait Evenness) is positive.

## S4.3: Structural Equation Models

*Saturated SEMs used in the main analyses*

In our main analyses, we constructed four saturated SEMs: a Bottom-up and a Top-down Control Model for explaning trait expansion patterns and a Bottom-up and a Top-down Control model for explaining trait dispersion patterns. In both saturated trait expansion models, we included the following variables: annual amount of rainfall, fire frequency, ReTR of the grass traits (canopy height, SLA and LA) and ReTR of the grasshopper traits (body length and relative wing length). In both the saturated, we included the ReTE variables instead of the ReTR variables of the same grass and grasshopper traits.

In all saturated SEMs, fire frequency was affected by annual rainfall. Furthermore, all grass and grasshopper traits were affected by both annual rainfall and fire frequency. In the saturated Bottom-up models, all grasshopper ReTR or ReTE variables were also affected by all ReTR or ReTE variables respectively, while these relations were vice versa in the Top-down Models. Fig. S4G en S4H show the saturated Bottom-up and Top-down models.

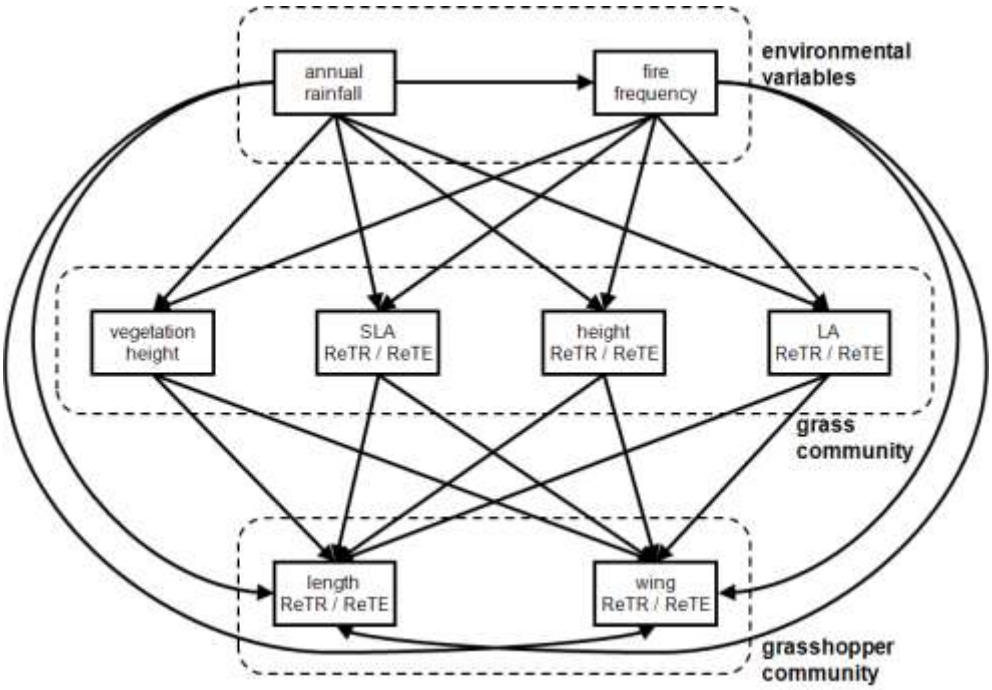


**Figure S4G.** Saturated Bottom-up model explaining variation in trait similarity patterns across sites. Note that we made separate Bottom-up Models for trait expansion and dispersion patterns.

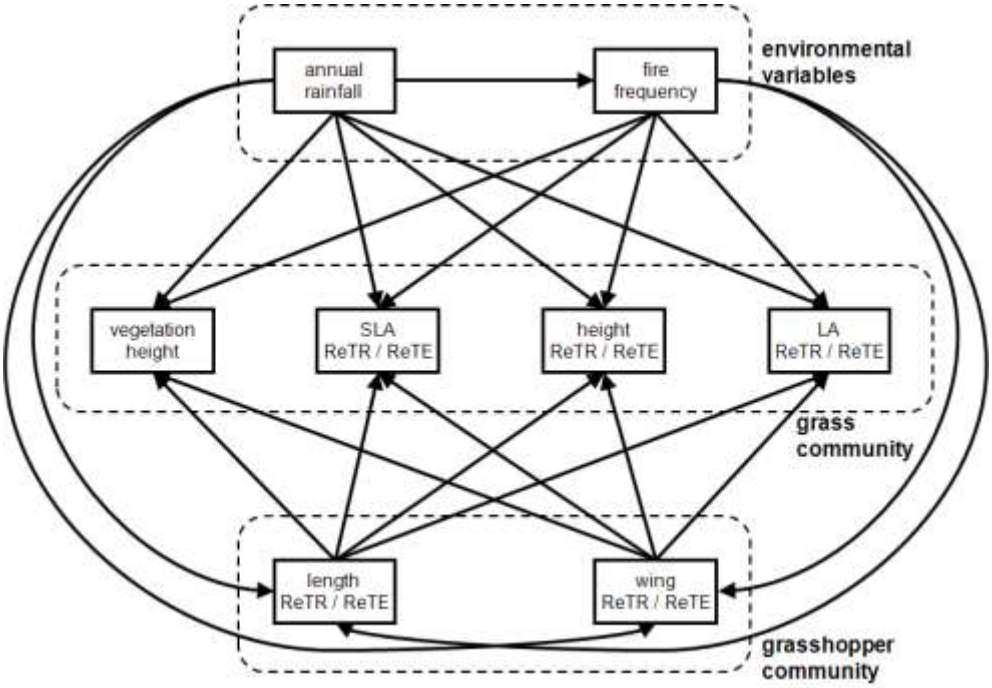


**Figure S4H.** Saturated Top-down model explaining variation in trait similarity patterns across sites. Note that we made separate Top-down Models for trait expansion and dispersion patterns.

*From saturated to final models: model pruning procedure*

The SEMs were run with the Amos 17.0 (Arbuckle 2007) software package using a maximum likelihood approach. Starting with the saturated saturated models described above, we followed the following iterative procedure to end up at our final models:

1. If possible, we added correlations that improved model fit, with the highest modification index values above the threshold value of 4.0.
2. If possible, we omitted the path with the least significant *P* value (T-test), until only *P* values below 0.05 were left in the model.
3. When a certain variable in the model was not causally connected with any other variable in the model, it was omitted.

Our final model only contained significant paths between the variables. Each variable had at least one causal link with another variable. Overall model fit was assessed using the $\chi^2$ statistic and the associated *P*-value. Model fit was considered adequate if the associated *P*-value was above 0.05 (Lee 2007), meaning that the null model (i.e. the final SEM model) could not be rejected. AIC values were used to compare the competing Bottom-up and Top-down Control Models.

*Trait expansion and dispersion patterns – in separate models or not?*

Non-random trait range patterns in natural communities are expected to result from abiotic stress filters (e.g. drought, heat or toxins), that only allow species with certain traits to end up in local communities (Weiher & Keddy 1995a; Weiher et al. 1998; Cornwell et al. 2006; Cornwell & Ackerly 2009). Competitive exclusion of species with similar niches and the consequent resource partitioning, results in communities of species with a high spacing of trait values that contribute to niche differences (Hutchinson 1957; Macarthur & Levins 1967; Pacala & Tilman 1994; Cornwell & Ackerly 2009). Different mechanisms are therefore affecting non-random trait dispersion and expansion patterns and therefore we did not have strong *a priori* expectations that these different properties of within-community trait distributions would be linked. That was the first reason why we

chose to separate our saturated SEMs describing the causes of trait expansion and dispersion patterns in our main analyses.

The second reason why we chose to separate our saturated SEMs describing the causes of trait expansion and dispersion patterns in our main analyses, is that with the complexity of the model, one increases the risk of overfitting, i.e. the risk that a certain model will describe the random error in the data, rather than underlying mechanisms of patterns that are generalizable (Grace 2006).

*Final SEM models with statistics of single paths*

Here, the trimmed SEMs are presented again, together with the associated statistics of all individual paths in them. Paths are numbered, and their correlation coefficients, *t*-values and *P*-values are given below Fig. S4I and S4J.

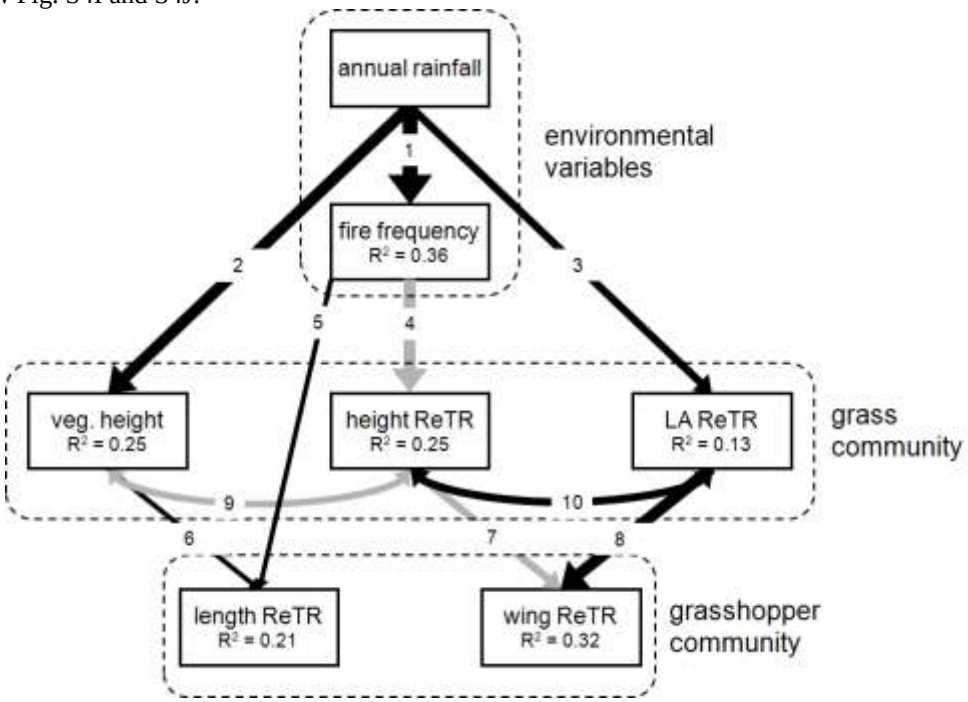


**Figure S4I**: Final model ($\chi^2$ = 5.957, d.f. = 11, *P* = 0.876, AIC = 39.957) explaining the trait range expansion of grasses and grasshoppers, after the non-significant paths were omitted. Single-arrow connectors represent direct effects of one variable on another, while double-arrow connectors represent correlations. The thickness of the arrows indicates the interaction strength. Black paths indicate positive effects, while gray paths indicate negative effects. The *R*-square indicates the total proportion of variation of the given variable that could be explained by the model.

Individual paths:

1. $r$ = 0.60, $t$ = 5.300, $P$ < 0.001
2. $r$ = 0.50, $t$ = 4.199, $P$ < 0.001
3. $r$ = 0.35, $t$ = 2.776, $P$ = 0.005
4. $r$ = -0.50, $t$ = -4.425, $P$ < 0.001
5. $r$ = 0.28, $t$ = 2.062, $P$ = 0.039
6. $r$ = 0.29, $t$ = 2.164, $P$ = 0.030
7. $r$ = -0.37, $t$ = -3.082, $P$ = 0.002
8. $r$ = 0.51, $t$ = 4.244, $P$ < 0.001
9. $r$ = -0.30, $t$ = -2.151, $P$ = 0.031
10. $r$ = 0.36, $t$ = 2.495, $P$ = 0.013

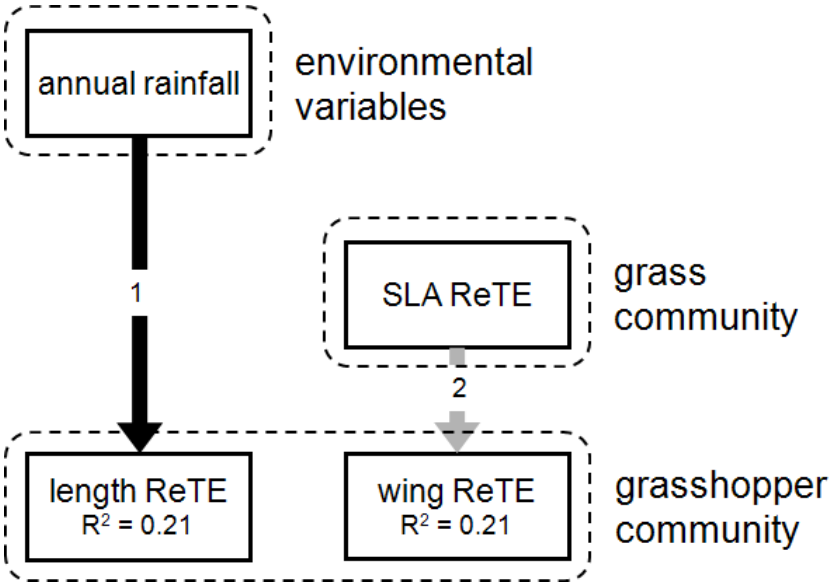


**Figure S4J:** Final model ($\chi^2$ = 2.582, d.f. = 4, $P$ = 0.630, AIC = 14.582) explaining the trait dispersion of grasses and grasshoppers, after the non-significant paths were omitted. Single-arrow connectors represent direct effects of one variable on another, while double-arrow connectors represent correlations. The thickness of the arrows indicates the interaction strength. Black paths indicate positive effects, while gray paths indicate negative effects. The *R*-square indicates the total proportion of variation of the given variable that could be explained by the model.

Individual paths

1. $r$ = 0.45, $t$ = -3.610, $P$ < 0.001
2. $r$ = -0.46, $t$ = 3.566, $P$ < 0.001

*Testing for robustness of the final SEMs of the main analyses: a full model*

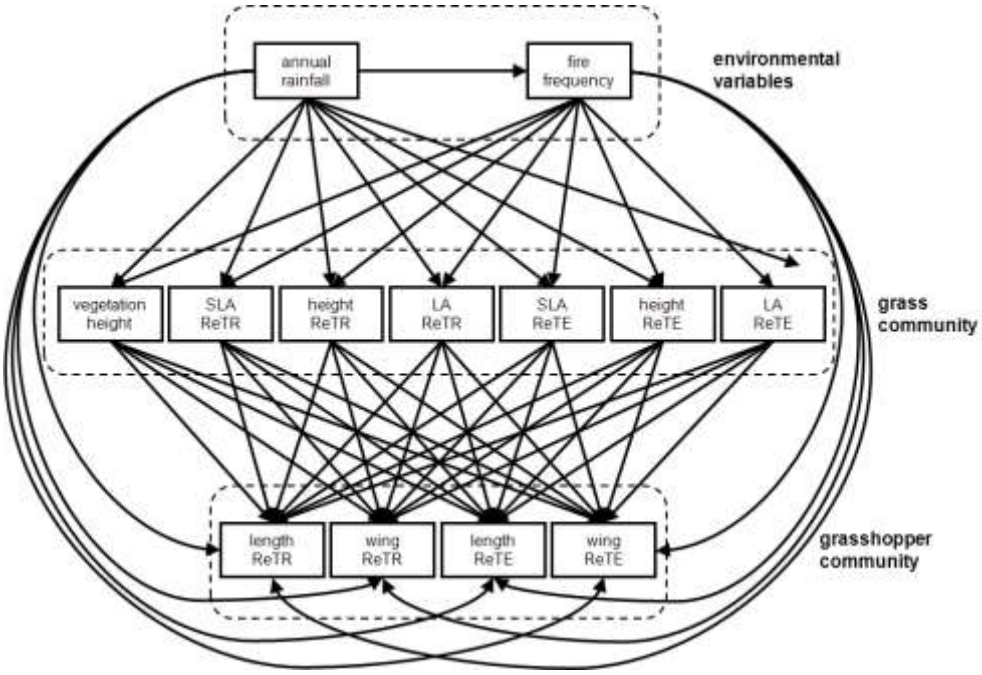


**Figure S4K.** Saturated Bottom-up model explaining variation in both trait expansion and dispersion patterns across our study area.

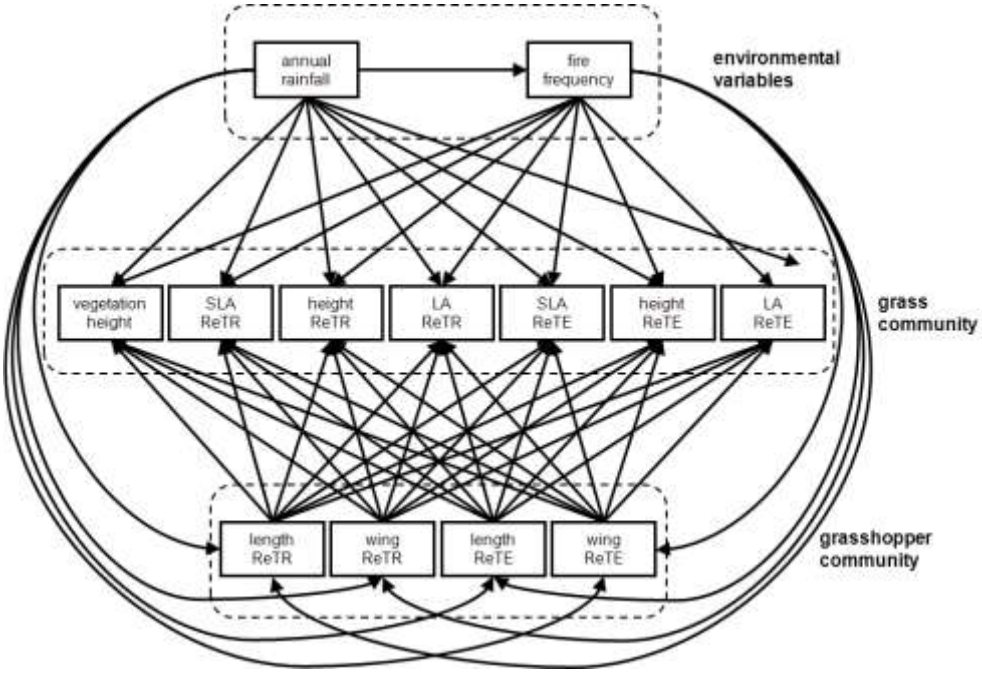

**Figure S4L.** Saturated Top-down model explaining variation in both trait expansion and dispersion patterns across our study area.

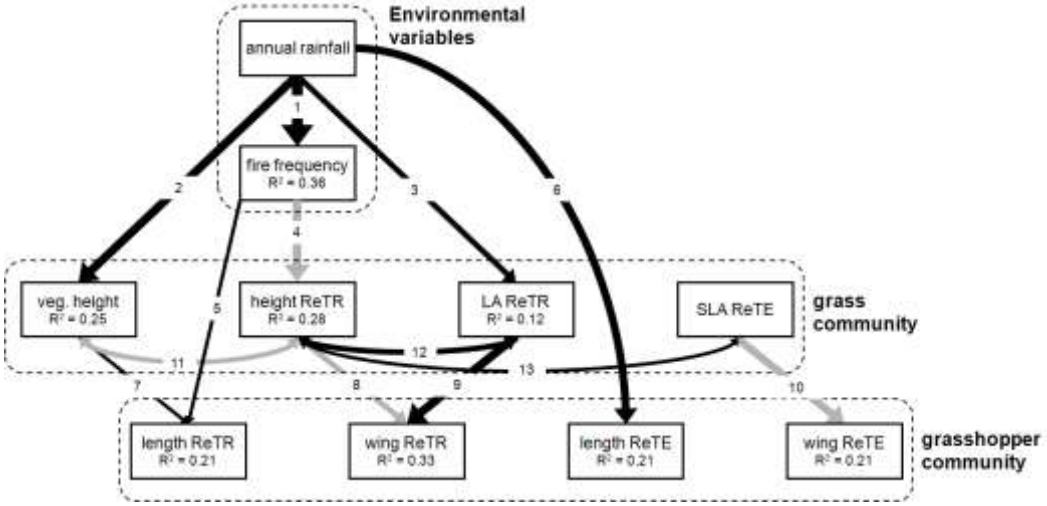


**Figure S4M:** Final model ($\chi^2$ = 22.099, d.f. = 32, $P$ = 0.905, AIC = 68.099) explaining the trait expansion and dispersion of grasses and grasshoppers, after the non-significant paths were omitted. Single-arrow connectors represent direct effects of one variable on another, while double-arrow connectors represent correlations. The thickness of the arrows indicates the interaction strength. Black paths indicate positive effects, while gray paths indicate negative effects. The *R*-square indicates the total proportion of variation of the given variable that could be explained by the model.

Individual paths

1. $r$ = 0.60, $\chi^2$ = 5.300, $P$ < 0.001
2. $r$ = 0.50, $\chi^2$ = 4.206, $P$ < 0.001
3. $r$ = 0.35, $\chi^2$ = 2.758, $P$ = 0.006
4. $r$ = -0.53, $\chi^2$ = -4.895, $P$ < 0.001
5. $r$ = 0.28, $\chi^2$ = 2.061, $P$ = 0.039
6. $r$ = 0.45, $\chi^2$ = 3.566, $P$ < 0.001
7. $r$ = 0.29, $\chi^2$ = 2.166, $P$ = 0.030
8. $r$ = -0.37, $\chi^2$ = -3.097, $P$ = 0.002
9. $r$ = 0.50, $\chi^2$ = 4.262, $P$ < 0.001
10. $r$ = -0.46, $\chi^2$ = -3.610, $P$ < 0.001
11. $r$ = -0.27, $\chi^2$ = -2.033, $P$ = 0.042
12. $r$ = 0.34, $\chi^2$ = 2.440, $P$ = 0.015
13. $r$ = 0.28, $\chi^2$ = 2.079, $P$ = 0.038

## S4.4: Multiple Regression analyses to identify the causes of variation in within-community trait similarity patterns across sites

To test whether our multitrophic framework of trait-based community assembly really yields to additional or different conclusions about the drivers of within-community trait similarity patterns than more traditional approaches, we also used Multiple Regression Analyses. The analyses were used to check what the most significant environmental drivers of within-community trait similarity patterns were when using a traditional approach (see for other examples of this or similar approaches: Pausas & Verdu 2008; Cornwell & Ackerly 2009). Multiple Regression Models were run with the following procedure:

1. A full model was developed with the within-community expansion or disersion in values of one grass- or grasshopper traits and rainfall, fire frequency and their interaction effect as the predictor variables.
2. Using a stepwise model selection procedure, we selected the model with the lowest AIC value.

*Results*

**Table S4A:** Outcome of Multiple Regression Analyses

| Response variable | predictors best model | *T* | *P* | Total $R^2$ |
|---|---|---|---|---|
| LA ReTR | rainfall | 4.366 | < 0.001 | 0.284 |
| SLA ReTR | | | | |
| height ReTR | rainfall + fire frequency + rainfall x fire frequency | 2.265; 2.557; -2.526 | 0.028; 0.014; 0.015 | 0.162 |
| body length ReTR | fire frequency | 2.570 | 0.013 | 0.121 |
| wing length ReTR | rainfall | 2.371 | 0.022 | 0.105 |
| LA ReTE | rainfall | -1.472 | 0.148 | 0.043 |
| SLA ReTE | - | | | |
| height ReTE | - | | | |
| body length ReTE | rainfall | 3.530 | < 0.001 | 0.206 |
| wing length ReTE | - | | | |

## S6.1: Study area

Field work was carried out in Hluhluwe-iMfolozi Park (HiP), an 89,665 ha mesic savanna nature reserve in Kwazulu-Natal, South Africa (Fig. S4A). Altitude ranges from 40 to 750 m above sea level (Fig. S4A). Most areas in the park frequently burn, with a mean fire return period of 3.8 years (Balfour & Howison 2002).

Vegetation types vary from grasslands, savannas, broad leaved thickets, to upland forest. Broad leaved woodland communities are dominated by *Euclea divinorum* or *Spirostachys africana,* but more than half of the park is savanna dominated by *Acacia* spp., with varying amounts of woody coverage (Brooks & MacDonald 1983; Whateley & Porter 1983).

*Site selection: mound and control sites*

Sixteen sites were selected stratified at random at different locations within HiP, within reach of a road and staying away from larger rivers, and avoiding drainages (Fig. S6A). All sites were at least 600 m apart from each other, which is far enough to consider different sites as independent replicates. Many plots were much further apart than this, with an average distance between nearest neighbour sites being 3.430 km. No plots are located in the most southern part of the park, which is managed as a wilderness area and therefore is not accessible for research.

*Site selection: browser preference assessment*

In June and July 2003 and 2004, tree communities in a total of 219 plots of 50 x 50 m, which were situated within all the different habitat types of HiP as identified by Whately & Porter (1983), except for grassland, were monitored. Distance between adjacent plots ranged between 100 and 3410 m (Fig. S6B). These distances were expected to be large enough to consider different plots as independent samples. Sampling effort for different habitat types was directly proportional to habitat contribution of the total area of HiP (Fig. S6B).

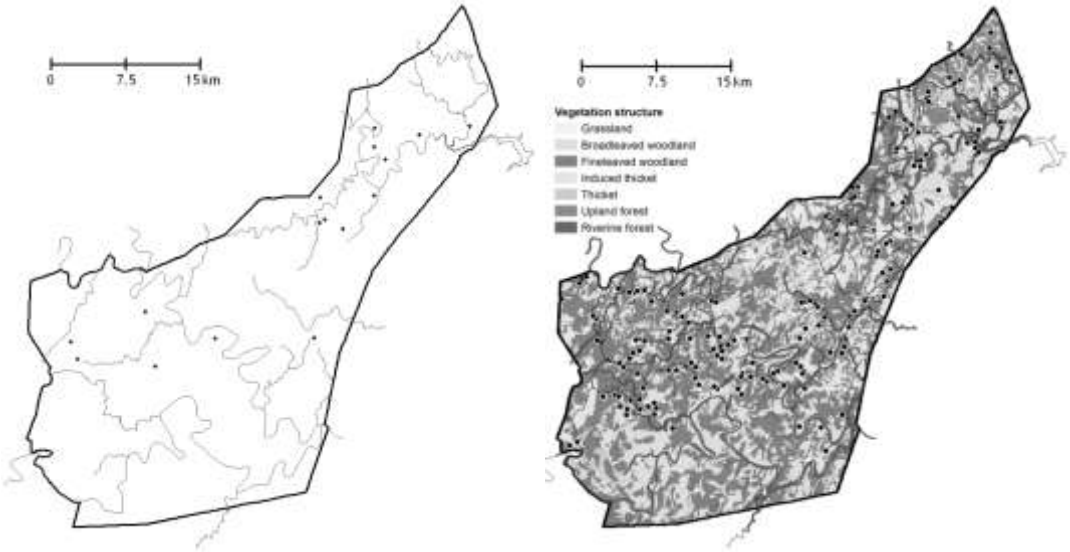


**Figure S6A**. A map (left) showing the sixteen different sites where *Macrotermes* mound and control tree communities were surveyed. **Figure S6B.** A map (right) showing the 219 different sites where trees were surveyed and inspected for damage by browsers.

## S6.2: Comparing trait variation within and between species

In order to assess whether most trait variation could be explained by differences in values between species or within species, between the habitat type (termite mound or control savanna), for each trait, an analysis of variance was used. Because we did not have trait values measured on mounds for most species, we only put five species in this analysis, which were the only species with trait values on both at least two control plots and two mound plots: *Berchemia zeyheri*, *Ehretia rigida*, *Euclea racemosa*, *Schotia brachypetala* and *Sideroxylon inerme*. These were all non-spiny species and therefore we omitted the analyses for spine length and spine density. $\eta^2$ Values were calculated to estimate the proportion of total trait variance explained by species differences and differences within species between origin.

**Table S6A.** ANOVA results for explaining trait variation among species and within species between location type (termite mound or control area). P values below 0.05 and $\eta^2$ values above 0.25 (i.e. more than 25 % percent of total variance explained by the given factor) are shown in bold. Abbreviations: SLA = specific leaf area; LA = individual leaf area, LFD = leaf fractal dimension, BA = branch angle, TD = leaf trichome density; WD = wood density; N, S, P, K, Na, Ca, Mg, Fe, Mn, Zn, B and Cu: leaf nitrogen, sulphur, phosphous, potassium, sodium, calcium, magnesium, zink, borium and cupper concentration respectively.

| Trait | Tot. SS | Resid. SS | Orig. SS | F | P | $\eta^2$ | Spec. SS | F | P | $\eta^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| SLA | 1.13E+05 | 5.37E+04 | 7.57E+02 | 0.366 | 0.550 | 0.007 | 5.84E+04 | 7.07E+00 | **0.001** | **0.518** |
| LA | 7.27E+03 | 5.10E+03 | 1.69E+01 | 0.083 | 0.776 | 0.002 | 2.15E+03 | 2.63E+00 | 0.058 | **0.296** |
| LFD | 1.39E+01 | 1.02E+01 | 3.66E-01 | 0.895 | 0.350 | 0.026 | 3.32E+00 | 2.03E+00 | 0.121 | 0.239 |
| BA | 6.89E+03 | 5.56E+03 | 3.34E+02 | 1.500 | 0.232 | 0.048 | 9.96E+02 | 1.12E+00 | 0.370 | 0.144 |
| TD | 1.42E+03 | 1.22E+02 | 1.87E+01 | 1.381 | 0.270 | 0.013 | 1.28E+03 | 2.37E+01 | **0.000** | **0.901** |
| WD | 1.21E+00 | 9.95E-01 | 1.52E-01 | 3.504 | 0.074 | 0.125 | 6.31E-02 | 3.65E-01 | 0.831 | 0.052 |
| N | 1.67E+01 | 5.94E+00 | 1.74E+00 | 8.186 | **0.008** | 0.104 | 9.06E+00 | 1.07E+01 | **0.000** | **0.541** |
| S | 3.55E+04 | 1.00E+04 | 8.85E+02 | 1.588 | 0.224 | 0.025 | 2.46E+04 | 1.10E+01 | **0.000** | **0.692** |
| P | 1.48E+04 | 5.86E+03 | 5.85E+02 | 1.796 | 0.197 | 0.039 | 8.37E+03 | 6.43E+00 | **0.002** | **0.565** |
| K | 1.34E+06 | 3.65E+05 | 7.26E+03 | 0.359 | 0.557 | 0.005 | 9.64E+05 | 1.19E+01 | **0.000** | **0.722** |
| Na | 2.39E+05 | 3.39E+04 | 5.48E+03 | 2.915 | 0.105 | 0.023 | 2.00E+05 | 2.66E+01 | **0.000** | **0.836** |
| Ca | 6.13E+05 | 5.09E+05 | 2.34E+04 | 0.830 | 0.375 | 0.038 | 8.06E+04 | 7.13E-01 | 0.594 | 0.132 |
| Mg | 1.70E+05 | 5.10E+04 | 3.00E+00 | 0.001 | 0.973 | 0.000 | 1.19E+05 | 1.05E+01 | **0.000** | **0.700** |
| Fe | 4.98E+07 | 3.06E+07 | 2.70E+06 | 1.592 | 0.223 | 0.054 | 1.65E+07 | 2.43E+00 | 0.086 | **0.331** |
| Mn | 1.81E+07 | 9.87E+06 | 3.12E+06 | 5.696 | **0.028** | 0.172 | 5.13E+06 | 2.34E+00 | 0.094 | **0.283** |
| Zn | 4.19E+05 | 1.45E+05 | 5.78E+03 | 0.718 | 0.408 | 0.014 | 2.68E+05 | 8.34E+00 | **0.001** | **0.640** |
| B | 1.87E+08 | 6.66E+07 | 1.63E+06 | 0.441 | 0.515 | 0.009 | 1.18E+08 | 7.99E+00 | **0.001** | **0.634** |
| Cu | 7.49E+04 | 4.80E+04 | 1.25E+03 | 0.476 | 0.503 | 0.017 | 2.57E+04 | 2.41E+00 | 0.087 | **0.343** |

The results show that in most traits there were significant differences in values among species. Origin (mound vs. control) did not explain a significant amount of variation of values in any of the traits. Furthermore, for most traits, species differences explained more than 25 % of the total variation and even up to 90.1 % (for trichome density), while origin (mound vs. control) did not explain more than 20 % of the variation in any of the traits. This suggests that trait variation can be best explained by intrinsic species differences and that genetic differences or phenotypic plasticity within species play a relatively minor role, supporting our choice to focus on intrinsic species values in our main analyses, rather than on variation within species.

## S6.3: PCA results

**Table S6B.** Proportion of trait variance explained by 10 most important PCA dimensions

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 |
|---|---|---|---|---|---|---|---|---|---|---|
| sd | 2.045 | 1.764 | 1.596 | 1.292 | 1.225 | 1.097 | 1.042 | 0.971 | 0.934 | 0.918 |
| prop. var | 0.190 | 0.141 | 0.116 | 0.076 | 0.068 | 0.055 | 0.049 | 0.043 | 0.040 | 0.038 |
| cum. prop | 0.190 | 0.332 | 0.447 | 0.523 | 0.591 | 0.646 | 0.695 | 0.738 | 0.778 | 0.816 |

**Table S6C.** Variable loadings (i.e. eigenfactors) of the PCA. Trait variables that had an $R^2$ above 0.20 with the given PC are given in bold. Abbreviations: SLA = specific leaf area; LA = individual leaf area, LFD = leaf fractal dimension; SL = spine length; SD = spine density; BA = branch angle, TD = leaf trichome density; WD = wood density; N, P, S, K, Na, Ca, Mg, Fe, Mn, Zn, B and Cu: leaf nitrogen, phosphous, sulphur, potassium, sodium, calcium, magnesium, zink, borium and cupper concentration respectively.

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 |
|---|---|---|---|---|---|---|---|---|---|---|
| SLA | **-0.359** | 0.013 | -0.121 | 0.239 | 0.085 | -0.093 | 0.056 | 0.069 | 0.349 | -0.039 |
| LA | 0.066 | **0.359** | -0.221 | -0.085 | -0.208 | -0.215 | 0.151 | 0.147 | -0.186 | 0.165 |
| LFD | -0.113 | **-0.427** | 0.071 | -0.144 | -0.105 | 0.122 | -0.210 | -0.352 | -0.052 | -0.074 |
| SL | -0.052 | -0.218 | 0.246 | -0.164 | 0.195 | -0.276 | 0.387 | 0.041 | -0.323 | -0.096 |
| SD | -0.072 | **-0.405** | 0.231 | -0.205 | -0.034 | -0.119 | 0.102 | 0.011 | -0.170 | -0.143 |
| BA | 0.007 | -0.087 | 0.147 | **0.475** | 0.095 | -0.246 | 0.315 | 0.150 | -0.234 | 0.214 |
| TD | 0.038 | -0.081 | **-0.301** | -0.263 | -0.290 | 0.280 | -0.020 | 0.232 | **-0.490** | 0.306 |
| WD | 0.050 | -0.069 | 0.221 | **-0.413** | 0.220 | -0.136 | -0.206 | 0.067 | 0.248 | **0.579** |
| PolC | 0.056 | -0.085 | 0.009 | 0.101 | -0.247 | **-0.615** | **-0.543** | 0.083 | -0.082 | -0.096 |
| C | **0.320** | -0.213 | -0.241 | 0.022 | 0.237 | 0.101 | -0.084 | -0.164 | 0.008 | -0.133 |
| N | **-0.320** | **-0.306** | -0.132 | -0.019 | -0.008 | 0.079 | 0.075 | 0.200 | -0.047 | -0.146 |
| P | **-0.271** | -0.174 | -0.265 | 0.157 | -0.083 | 0.119 | -0.072 | 0.217 | -0.087 | -0.004 |
| S | **-0.304** | 0.104 | 0.226 | 0.034 | -0.266 | -0.041 | -0.284 | 0.039 | -0.097 | -0.110 |
| K | **-0.386** | -0.017 | -0.162 | -0.059 | -0.037 | -0.045 | 0.102 | 0.157 | 0.250 | 0.087 |
| Na | -0.170 | **0.269** | -0.018 | 0.154 | 0.310 | -0.071 | -0.198 | -0.297 | -0.464 | -0.022 |
| Ca | -0.205 | 0.199 | **0.371** | -0.068 | -0.059 | 0.212 | 0.158 | -0.100 | -0.019 | -0.111 |
| Mg | -0.090 | 0.173 | **0.412** | 0.067 | -0.347 | 0.231 | -0.011 | -0.110 | -0.039 | 0.156 |
| Fe | -0.163 | 0.186 | 0.055 | **-0.363** | 0.302 | -0.174 | -0.012 | 0.274 | -0.023 | -0.076 |
| Mn | 0.190 | 0.221 | -0.140 | -0.292 | -0.212 | -0.147 | 0.216 | -0.095 | 0.064 | -0.461 |
| Zn | **-0.293** | 0.060 | -0.172 | -0.282 | -0.159 | -0.187 | 0.102 | -0.239 | 0.032 | -0.045 |
| B | -0.150 | 0.200 | -0.002 | -0.101 | **0.409** | 0.251 | -0.308 | 0.204 | -0.181 | -0.230 |
| Cu | **-0.262** | 0.038 | -0.271 | -0.028 | 0.110 | -0.127 | 0.091 | **-0.572** | -0.080 | 0.288 |

## S7.1: Trait information grasses

**Table S7.1A.** Average trait values of each grass species. Species abbreviations: Cyn dac = *Cynodon dactylon*; Dac aus = *Dactyloctenium australis*; Dig lon = *Digitaria longiflora*; Era cur = *Eragrostis curvula*; Set sph = *Setaria sphacelata*; Spo pyr = *Sporobolus pyramidalis*. Trait abbreviations: evap. w. = averaporation of week…; AG = absolute growth; RGR = relative growth rate; WUE = water use efficiency; RLAR d/r= relative leaf accumulation rate drought / recovery period; RWF = root weight fraction; DRWF = deep root weight fraction; SRL = specific root length.

| Trait | lawn species | | | | | | bunch species | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Cyn dac* | | *Dac aus* | | *Dig lon* | | *Era cur* | | *Set sph* | | *Spo pyr* | |
| | c | d | c | d | c | d | c | d | c | d | c | d |
| evap.w. 4 (mL $day^{-1}$) | 11.22 | 8.35 | 11.03 | 6.82 | 4.14 | 3.37 | 6.48 | 5.89 | 9.67 | 9.56 | 5.20 | 3.34 |
| evap.w. 7 (mL $day^{-1}$) | 24.83 | 0.10 | 20.15 | 0.06 | 9.15 | 0.79 | 18.57 | 0.73 | 18.23 | 0.01 | 10.87 | 1.95 |
| evap.w. 9 (mL $day^{-1}$) | 30.45 | 17.29 | 25.95 | 20.10 | 9.79 | 7.90 | 24.34 | 14.73 | 21.40 | 15.66 | 14.05 | 6.17 |
| AG (g) | 10.82 | 4.42 | 7.56 | 3.91 | 4.07 | 2.57 | 7.75 | 4.42 | 9.00 | 5.23 | 6.14 | 3.06 |
| RGR (g $g^{-1}$ $day^{-1}$) | 0.048 | 0.034 | 0.045 | 0.038 | 0.038 | 0.033 | 0.036 | 0.032 | 0.038 | 0.031 | 0.030 | 0.022 |
| WUE (g $mL^{-1}$) | 0.011 | 0.012 | 0.010 | 0.011 | 0.011 | 0.013 | 0.011 | 0.013 | 0.012 | 0.014 | 0.013 | 0.016 |
| RLAR d. (leaf $leaf^{-1}$ $day^{-1}$) | 0.066 | 0.033 | 0.062 | 0.031 | 0.066 | 0.043 | 0.040 | 0.020 | 0.042 | 0.018 | 0.035 | 0.030 |
| RLAR r. (leaf $leaf^{-1}$ $day^{-1}$) | 0.031 | 0.035 | 0.030 | 0.055 | 0.043 | 0.056 | 0.018 | 0.038 | 0.014 | 0.036 | 0.037 | 0.029 |
| RWF | 0.24 | 0.18 | 0.11 | 0.14 | 0.16 | 0.19 | 0.24 | 0.26 | 0.36 | 0.36 | 0.27 | 0.26 |
| DRWF | 0.52 | 0.55 | 0.70 | 0.65 | 0.60 | 0.57 | 0.55 | 0.58 | 0.56 | 0.53 | 0.58 | 0.52 |
| SRL (m $g^{-1}$) | 59.69 | | 51.60 | | 44.85 | | 22.38 | | 13.28 | | 41.29 | |

## S8.1: Environmental gradients in HiP

The average annual amount of rainfall in HiP during the period 2001-2007 ranged from less than 500 mm in the south to more than 800 mm in the north (Fig. S8A). Plots were partially spread out over this gradient, with the driest plot receiving 548 mm per year, the wettest plot receiving 837 mm per year and an average amount of annual rainfall across all plots being 674 mm per year.

Another important environmental factor in HiP is the gradient in fire frequency (Fig. S8B). Fires in HiP are mostly ignited by park managers. Fire frequency, as measured over the period 1956-2004, ranges from less than 5 to over 25 in HiP. The plots investigated in this study covered most of this range, with the plot with lowest fire frequency experiencing 8 fires over this period, the plot with highest fire frequency experiencing 24 fires over the period and an average fire frequency across plots of 14.8 (Fig. S8B).

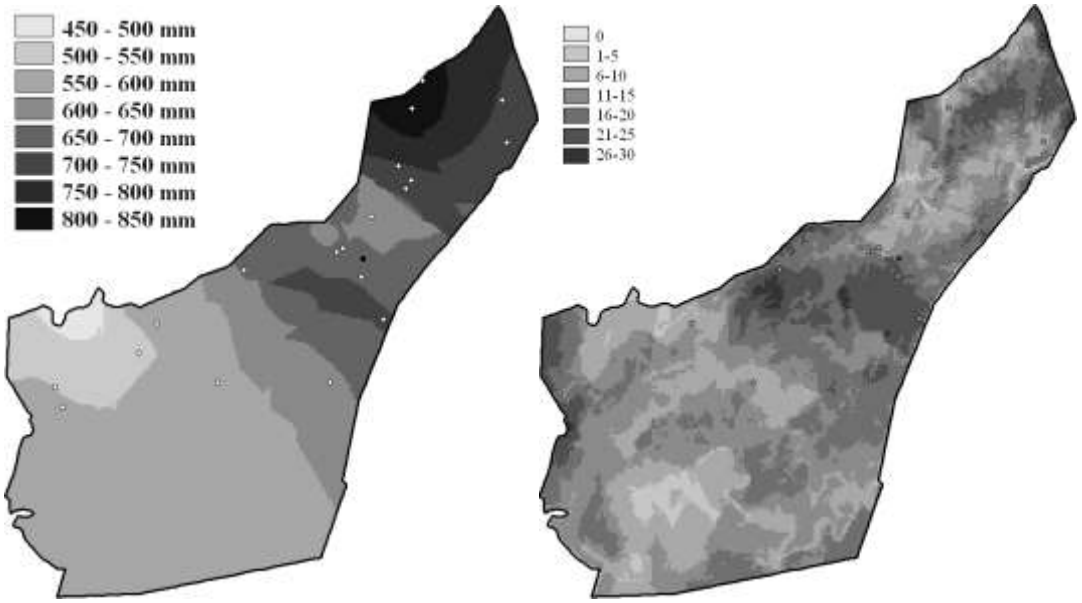


**Fig. S8A.** An average amount of annual rainfall (in mm) map of HiP. The dots represent the plots visited fort his study, with the purple dot representing plot nr. 17, used for robustness checks in this study, because it was considered the most 'representative' plot. Fig. S8B. A fire frequency map of HiP, showing the number of times different locations in the park were burnt in the period of 1956-2004. The dots represent the plots visited fort his study, with the dark dot representing plot nr. 17, used for robustness checks in this study, because it was considered the most 'representative' plot.

## S8.2: Community Assembly Simulation (CAS) models: how do FD values respond to relative contributions of different community assembly processes and to a change in the order of these processes?

In our main analyses, we ran CAS models with different community assembly processes in the following order: (1) random removal, (2) filtering or (3) limiting similarity. As such, we assumed a certain order in community assembly, which is most in line with other literature on this topic (e.g. Cornwell & Ackerly 2009; Van der Plas et al. 2012), although one might argue that *a priori* assumptions about the order of community assembly processes are risky, because such assumptions could potentially bias model outcomes. To test this, here, we ran several CAS models differing in two ways from each other: (1) in the relative contribution of different community assembly steps, and (2) in the order of these community assembly processes.

*(1) relative contributions of different community assembly processes*

Parameter space of the different contributions of community assembly processes was explored with 0.05 intervals. Hence, we ran models with parameter settings of 1-0-0, 0.95-0.05-0, 0.95-0-0.05, 0.9-0.1-0, 0.9-0.05-0.05, etc., in which the first number stands for the relative contribution of random removal steps in the model, the second number for the relative contribution of filtering steps and the last number for the relative contribution of random removal steps with a total number of 231 (= $\frac{(\frac{1}{0.05}+1)\cdot(\frac{1}{0.05}+2)}{2}$) different models, with 1000 replicates for each one. For each species set resulting from these CAS models, we calculated FRic, FEve and FDiv and we calculated the average of the 1000 replicate FRic, FEve and FDiv for of the 231 different CAS models. The results of this plus the interpretations can be found in the main document (Fig. 3).

*(2) a change in the order of these processes*

Secondly, we investigated for each of these parameter combinations how the order of community assembly processes changed the FRic, FEve and FDiv values of resulting communities. This was done by running models with each of the 231 different parameter combinations described above, but with different orders of processes: (1) random removal steps first, filtering second and finally limiting similarity (as done in the main analyses), (2) random removal, filtering and limiting similarity steps in separate blocks, but the order of these blocks randomized over replicates (3) the order of random removal, filtering and limiting similarity steps completely randomized. As such, we thus ran a total of 231 x 3 = 693 different CAS models, with 1000 replicates for each model, and we investigated (i) whether the order of different community assembly processes had large absolute effects on functional diversity values of resulting communities and (ii) whether responses of functional diversity values to different relative contributions of different community assembly steps in models depended on the order of these community assembly steps.

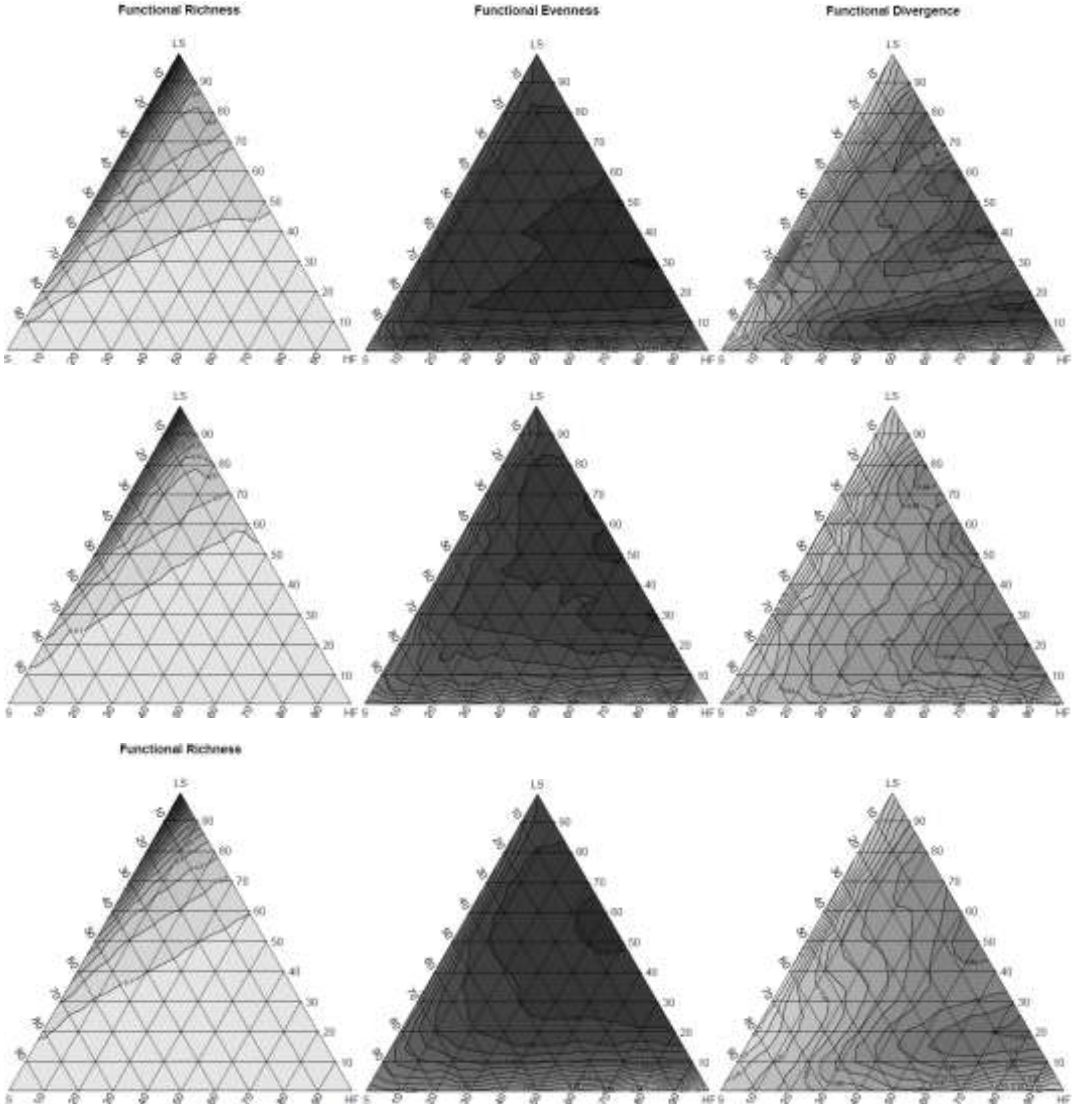

**Fig. S8C.** Ternary plots with the responses of FRic (left), FEve (middle) and FDiv (right) to changes in the relative contribution of random removal, filtering and limiting similarity steps in CAS models applied to plot 17. The upper row shows results for CAS models in which random removal steps were run first, then filtering steps, then limiting similarity steps. The middle row shows results for CAS models with random removal, filtering and limiting similarity steps in separate blocks, but the order of these blocks randomized over replicates. The bottom row shows results for CAS models with the order of random removal, filtering and limiting similarity steps completely randomized. The darker the colour, the higher the FD value.

As can be seen in Fig. S8C, changing the order in which different community assembly processes take place does not lead to very large changes in FD values. In all scenarios, FRic values are highest when limiting similarity processes dominate and lowest when filtering processes dominate in community assembly. FEve and FDic values are highest when stchasicity is close to zero, and community assembly is regulated by a combination of filtering and limiting similarity processes.

## S8.3: More details about how the ABC-SMC approach was used to infer the relative contributions of different processes in community assembly.

Model selection was performed through the method of Approximate Bayesian Computation (ABC) within a Sequential Monte Carlo (SMC) framework (Toni et al. 2009; Beaumont 2010; Hartig et al. 2011). With ABC, model selection is performed through the comparison of one or more summary statistics of observed data with those generated by models. This is in contrast to typical Maximum Likelihood optimization where the likelihood of different competing models is optimized. For our CAS models no likelihood could be calculated and therefore we relied on the use of four summary statistics: FRic, FEve, FDiv and CAWTA values. Before model selection, we standardized all summary statistics to a mean of zero and a standard deviation of one, so that each different summary statistic had the same impact on the fitting procedure. The fit of CAS models was calculated as:

$$Fit_{total} = Fit_{FRic} + Fit_{FEve} + Fit_{FDiv} + Fit_{CAWTA},$$

in which $Fit_{FRic}$, $Fit_{FEve}$ and $Fit_{FDiv}$ are the absolute difference between respectively FRic, FEve and FDiv values from the observed community and those generated by the CAS model, while $Fit_{CAWTA}$ is the (multidimensional) Euclidian distance between the 8 CAWTA values of the observed community and those generated by the CAS model. High $Fit_{total}$ values thus indicated poor fit, while low values indicated good fit. Using the fit of the models, importance resampling was performed largely following the algorithm described in Toni (2009), consisting of the following steps:

S1 Initialize vector of acceptance thresholds $\varepsilon_1 \ldots \varepsilon_T$, set population indicator $t = 0$

S2.0 Set the particle indicator $i = 1$

S2.1 If t = 0, sample $\theta^{**}$ independently from the prior $\pi(\theta)$.
Else, sample $\theta^{*}$ from the previous population with weights $w_{t-1}$ and perturb the particle using a normally distributed perturbation kernel $K$ (mean 0, standard deviation of σ) to obtain $\theta^{**}$

S2.2 If $\pi(\theta^{**}) = 0$, return to S 2.1.

S2.3 Simulate a candidate dataset $x^{*}$ using our CAS model with parameters $\theta^{**}$.

S2.4 If $Fit_{total} \geq \varepsilon_t$, return to S2.1

S2.5 Set $\theta_t^{(i)} = \theta^{**}$ and calculate the weight for particle $\theta_t^{(i)}$:
If t = 0, $w_t^{(i)} = 1$.
If t > 0, $w_t^{(i)} = \frac{1}{\sum_{j=1}^{N} w_{t-1}^{(j)} K_t(\theta_{t-1}^{(j)}, \theta_t^{(i)})}$
If i < N, set i = i + 1, go to S2.1

S3 Normalize the weights.
If t < T, set t = t + 1, go to S2.0.

We chose our acceptance threshold as an exponentially decreasing series, such that initially samples are easily accepted, but as *t* increases, the acceptance rate quickly decreases. Our prior consisted of the full [0, s] interval for the three parameters under investigation here: stochasticity, filtering and competition, in which s is the number of CAS model steps (= richness of species pool – richness of local community). Perturbation in step 2.1 was performed by randomly picking one of the three parameters and adding a value obtained from a normal distribution with mean 0 and standard deviation of 1. Because the three parameters together have to add up to the full community size, the difference obtained by the perturbation was corrected by subtracting or adding the same amount from the other two parameters. Whereas in Toni et al. (2009) weights are multiplied by the prior probability of the found parameter combination, in our case, this probability is always 1. As our prior distribution is a step function with a value of 1 in the interval [0, s] and a value of 0 outside this interval, any proposed parameter combinations outside the prior would have caused the algorithm in step 2.2 to start over again and propose a new combination.
The algorithm was repeated until the acceptance rate (S2.4) had diminished to 1 in a million particles. For each plot, we ran the algorithm ten times, to investigate whether the ABC model selection generated repeatable parameter values. The average parameter values of these ten models are reported in the 'results' section.

## S8.4: Community Assembly Simulation (CAS) models: how well can our ABC-SMC model selection procedure distinguish between competing models?

In order to investigate how well our ABC-SMC approach can distinguish between competing CAS models, we investigated three criteria: (1) how much variation is there in the parameter values from the posterior distribution? (2) does the ABC-SMC approach lead to repeatable patterns, that is: when the algorithm is run several times, are average parameter values of posterior distributions relatively constant across different runs for the same plot? And (3) does the reverse fitting of summary statistics generated by a CAS model lead to the selection of a model with similar parameter settings as the model that originally generated the summary statistics?

*How much variation is there in the parameter values from the posterior distributions?*
We applied the ABC-SMC model selection approach 10 times to each of the 20 plots we investigated in this study. Each time, the ABC-SMC model selection procedure generated a posterior distribution of parameter values from 'accepted models', i.e. CAS models that generated summary statistics that were similar enough to observed summary statistics to be considered 'adequate'. In the rest of our study, we reported and analyzed the average parameter values of these posterior distributions, but an interesting question is how much variation there is within this posterior distribution: ideally, this variation would be as low as possible, which would indicate that models with a small range of parameter values clearly fit the summary statistics better than competing models.
We used the coefficient of variation ($CV = \frac{sd_{parameter\ value}}{mean_{parameter\ value}}$) as a standardized measure for the variation of parameter values in the posterior distribution, with CV values below 1 indicating relatively low variation and CV values above 1 indicating relatively high variation. CV values for stochasticity steps ranged from 0.017 to 0.540, with an average of 0.152. CV values for filtering steps ranged from 0.177 to 1.217, with an average of 0.593. CV values for limiting similarity steps ranged from 0.146 to 21.967 (for plot 19), with an average of 2.123. When ignoring plot 19, CV values for limiting similarity steps ranged from 0.146 to 3.145, with an average of 1.080. In general, variation in parameter values from the posterior distribution of ABC-SMC algorithms was therefore reasonably low.

1. *Does the ABC-SMC approach lead to repeatable patterns?*

We applied the ABC-SMC model selection approach 10 times to each of the 20 plots we investigated in this study. So not only within posterior distributions there could be variation in parameter values, but also across replicate runs within the same plot, the average parameter value of posterior

distributions could differ. Also in this case, ideally, this variation should be as low as possible, which would indicate that the ABC-SMC approach leads to repeatable patterns.

We used the coefficient of variation ($CV = \frac{sd_{parameter\ value}}{mean_{parameter\ value}}$) as a standardized measure for the variation of average parameter values across replicate model selection runs, with CV values below 1 indicating relatively low variation and CV values above 1 indicating relatively high variation. CV values for stochasticity steps ranged from 0.002 to 0.308, with an average of 0.308. CV values for filtering steps ranged from 0.043 to 1.955, with an average of 0.344. CV values for limiting similarity steps ranged from 0.039 to 2.206, with an average of 0.407. In general, replicate model selection runs thus resulted in very similar average parameter values of posterior distributions. Therefore, we conclude that the ABC-SMC approach leads to repeatable patterns.

2. *Does the reverse fitting of summary statistics generated by a CAS model lead to the selection of a model with similar parameter settings as the model that originally generated the summary statistics?*

We performed the 'reverse fitting procedure' on plot 17. We used 'generator models' with certain parameter settings to generate summary statistics (FRic, FEve, FDiv and CAWTA values). With the ABC-SMC approach described in the main document, these summary statistics were used to identify a 'best fitting model'. We then compared each of the 3 parameter values of the 'generator models' with parameter values of the associated 'best fitting models': ideally, if our ABC-SMC approach works perfectly, parameter values of 'generator modes' and 'best fitting models' would be exactly equal. In case our ABC model selection procedure would be totally uninformative, leading to the selection of CAS models with random parameter settings, the average expected parameter deviation would be:

$$DEV_{exp} = \left(\frac{1}{3} - x + 2x^2 - \frac{2}{3}x^3\right) \cdot 100$$

in which x is the given parameter setting from the 'generator model' (e.g. the percentage of random removal steps in the 'generator model'). In total, we ran reversely fitted 10 x 231 'generator models': we ran 'generator models' with each of the 231 parameter settings described above, with ten replicates for each parameter combination.

On average, the deviation in parameter value of 'best fitting models' from the associated 'generator models' was 3.5 % for the relative proportion of random removal steps, 2.8 % for the relative proportion of filtering steps and 1.8 % for the relative proportion of limiting similarity steps. These deviations shifted slightly over parameter space (Fig. S8D). Average expected deviations were 27.7 % for each parameter value, and thus much higher than observed. This made us believe that generally, the ABC-SMC approach was adequate in distinguishing CAS models with different parameter combinations. Although expected parameter deviations shifted over parameter space (Fig. S8D), for each parameter combination, observed deviations between 'generator models' and associated 'best fitting models' were smaller deviations when assuming that our ABC-SMC approach would randomly select models (Fig. S8E), meaning that irrespective of the parameter combination of the 'generator model', the ABC procedure performed better than random. When 'generator models' had extreme parameter settings (i.e. the relative contribution of a given community assembly process being either close to 0 % or close to 100 %), the ABC model selection procedure performed relative better than in cases where parameter settings of 'generator models' were less extreme (Fig. S8E).

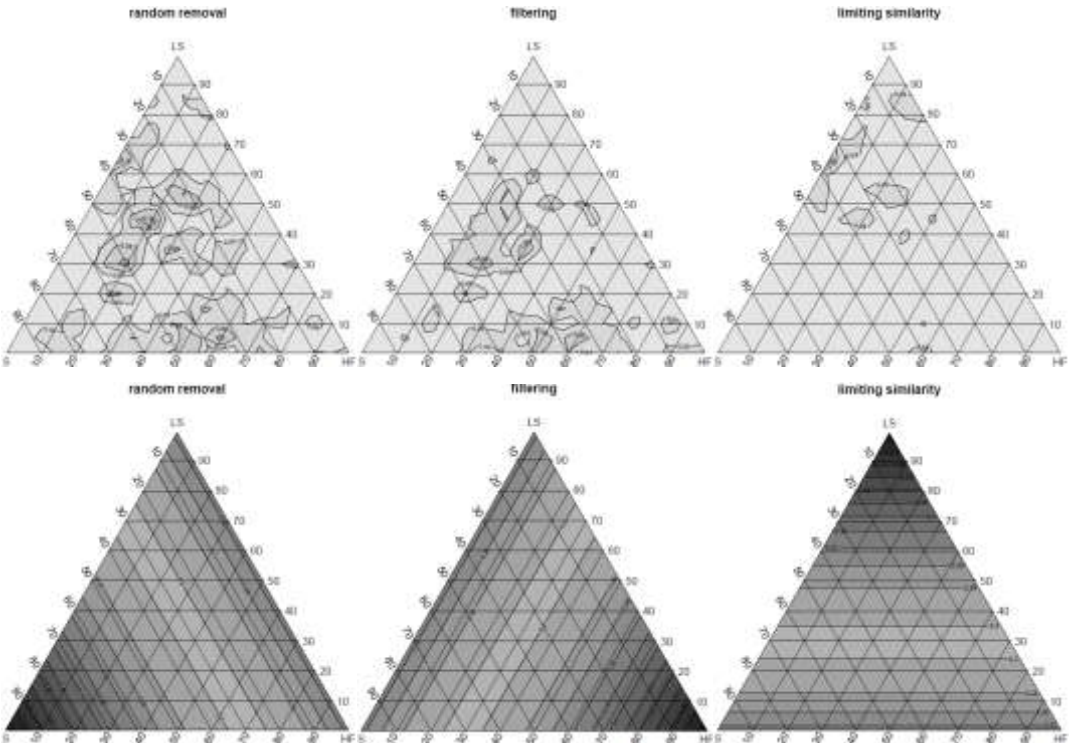


**Fig. S8D.** Ternary plots with on top the absolute deviation between parameter values from 'generator models' and their associated 'best fitting models', as a response to the parameter values of the 'generator models'. The three parameter deviations considered are: the relative contribution of (i) random removal steps (left), (ii) filtering steps (middle) and (iii) limiting similarity steps (right) in community assembly. Below one can see the expected deviation between parameter values from 'generator models' and their associated 'best fitting models', assuming that the ABC-SMC approach selects 'best fitting models' randomly over parameter space. Deviation values for positions in parameter space were estimated using bilinear interpolation. Abbreviations: RR = random removal, F = filtering and LS = limiting similarity.

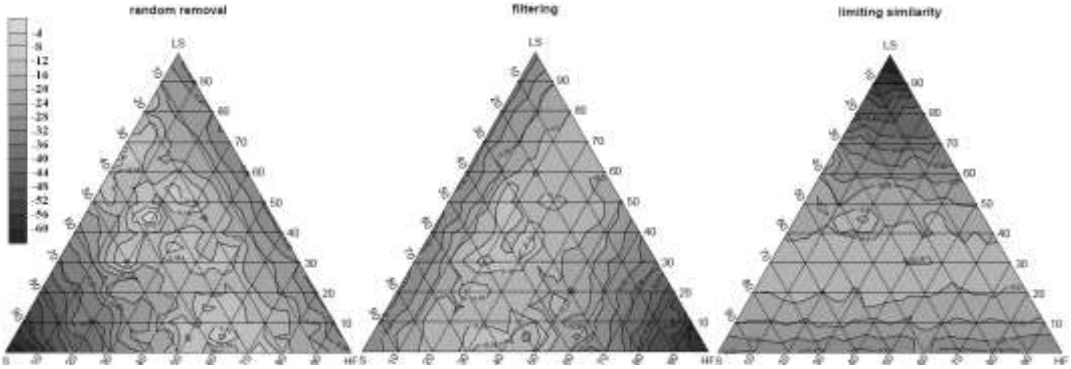


**Fig. S8E.** Ternary plots with the observed – expected deviation between parameter values from 'generator models' and their associated 'best fitting models', as a response to the parameter values of the 'generator models'. Note that values are always negative. Deviation values for positions in parameter space were estimated using bilinear interpolation. Abbreviations: RR = random removal, F = filtering and LS = limiting similarity.

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# English summary

Sets of co-occurring species, better known as 'communities', are the result of ongoing immigration, emigration, reproduction and extinction events. To a certain extent, these events are stochastic: for example, it is often very difficult, if not impossible, to predict whether a seed traveling by air will or will not end up at a certain location. At the same time, since the earliest studies on community ecology, people have also recognized that many events can be quite deterministic: you can be very sure that in an African savannah, grazers can be found, while humpback whales, mangrove trees and penguins cannot.

The reason why some species can (potentially) live at a particular location, and other species cannot, is because species differ from each other in 'functional traits'. Traits are measurable properties of an organism, such as body size, metabolic rate, growth rate, and leaf thickness, and they are considered 'functional' when they explain differences in performance (e.g. reproduction, survival) of organisms. While there is usually quite some variation of traits within species, trait variation between species is usually much larger. Indeed, this interspecific trait variation is usually used to identify different species. Many of these traits are not only informative about the identity of a species, but also about the type of environment in which it can occur. For example, to grow in a very dry environment, plants need to be very conservative with water, and one way to preserve water is by only growing very thick leaves, thereby limiting transpiration. As a result, in a desert, one will observe many succulent plant species, but hardly (if any) fine-leaved plant species. This is called 'abiotic filtering': aspects of the abiotic (i.e. not-living) environment (in this case: drought) limit certain species to occur at a particular location, but other species not.

In many cases, traits can thus be used to predict in which kind of environment a species can potentially occur. However, investigating which species are or are not 'filtered out' by the abiotic environment is not enough to understand the assembly of ecological communities. Species not only respond to their abiotic environment, but also to interactions with other species. One type of interaction that is often studied is competition. When two species, for example, the red (*Sciurus vulgaris*) and grey squirrel (*Sciurus carolinensis*), use similar resources, it often happens that one of the two species (here: the grey squirrel) will be more efficient in capturing the resource than the other. As a result, the other species (the red squirrel) will be outcompeted. Often traits can be used to predict to which extent species compete with each other: species compete when they use the same resources, and traits might tell us about the resource use of a species. In the example of red and grey squirrels, the possession of sharp incisors and similar digestive systems makes that both species specialize on seeds as their main food source. As a result, the more efficient grey squirrel species usually outcompetes the other.

When looking at abiotic filtering and resource competition and how these processes are mediated by traits, we thus observe an intriguing phenomenon: while species need to be similar to cope with the same abiotic environment, at the same time they need to be dissimilar enough (in traits related to resource type) to be able to co-occur. Traditionally, this conceptual idea has been used in studies on community assembly to detect the presence of three different community assembly processes. When (1) trait values of an observed set of co-occurring species have a smaller range than the values of a random set of species with the same richness (a pattern called trait underexpansion), this is usually attributed to abiotic filtering. When (2) the trait values of an observed set of co-occurring species are more evenly spaced than trait values of a random set of species with the same richness (a pattern called trait overdispersion), this is usually attributed to be the outcome of competition. Alternatively, when (3) both trait range and evenness values of observed communities are the same as in a 'random' community, it is likely that stochasticity is more important in driving community assembly.

This approach of inferring community assembly processes from trait distributions is intuitively very simple, which is probably one of the main reasons why it has received so much popularity over the

years. However, there are also some criticisms on this approach. A first one is that this trait-based approach on community assembly ignores a wide array of ecological processes that shape communities: among them, trophic interactions and ecosystem engineering. Also, usually communities are assembled by the interplay of different types of processes: a community is more likely to be assembled by *both* abiotic filtering, competition *and* stochastic processes than by *either* abiotic filtering, competition *or* stochasticity alone. In this thesis, I dealt with these criticisms: in African savannahs, I investigated how incorporation of (1) trophic interactions and (2) ecosystem engineering processes change our view on trait-based community assembly and I investigated how (3) the interplay of different types of processes affects the trait distribution patterns of co-occurring species.

In the first four chapters (2,3,4,5), I investigate the effects of trophic interactions on trait-based community assembly. In savannahs of Hluhluwe-iMfolozi Park (HiP), South-Africa, I studied how the community assembly of organisms of two trophic levels, grasses and (herbivorous) grasshoppers, are affected by the grazing of (1) ungulate herbivores (such as white rhino, zebra, buffalo and antelope species), (2) environmental gradients and (3) by community composition of the other trophic level. It appeared that grazers create mosaics of short and tall vegetation, with the shorter vegetation patches being dominated by small-leaved grass species and the taller patches being dominated by large-leaved grass species. As a result, at larger scales, grazing increases species richness of grasses. Also grasshopper communities are highly affected by grazing: grazed areas contain very different, usually smaller, grasshopper species than ungrazed areas. However, the species richness of grasshoppers is *not* affected by grazing, perhaps because the potential positive effects of a higher food type diversity (i.e. grass species richness) for grasshoppers are counterbalanced by the negative effects of a decreased food availability (vegetation height). Also, grazing appeared to have no effects on trait similarity patterns of co-occurring grasses and grasshoppers. To some extent, this was surprising. Intuitively, one might think that due to increased stress (defoliation), grazers might filter out grazing-intolerant species with particular traits (e.g. grass species with low growth rates), thereby causing underexpansion patterns in grass communities, with cascading effects on grasshopper communities. Possibly, the higher spatial turnover in community composition in grazed areas counterbalances this phenomenon: at larger scales, one finds both grazing tolerant species (in short, grazed patches) and grazing intolerant species (in tall vegetation patches). Although not responding to the grazing by ungulates, trait expansion patterns of grasses appeared to respond to larger scale environmental gradients: generally, grass traits are less underexpanded in areas with high rainfall and low fire frequencies, in line with classical expectations. Trait expansion patterns of grasshopper communities, on the other hand, hardly respond to environmental gradients, but instead respond to variation in vegetation height and to variation in trait expansion patterns of co-occurring grass communities. This thus shows that trait distribution patterns of one trophic level can be imprinted on a higher one, emphasizing how important tropic interactions can be in understanding community assembly. A review of the relevant literature showed that many of our observed effects of wild ungulate grazer communities on grass and grasshopper community assembly are consistent with other studies. In general, effects of grazers on species richness of plants are more positive (or less negative) than effects on species richness of arthropods. Additionally, many studies show that grazing usually favors small-sized arthropod species over larger ones, possibly because the former require smaller quantities of food (plant biomass), are less vulnerable to predation in uncovered (i.e. bare) patches, require higher microtemperatures to develop, or because of a combination of these factors.
In a last chapter on the effects of trophic interactions on community assembly, it is shown that in Serengeti National Park (Tanzania), rocky outcrops, or kopjes, form mini-islands in the savannah, with a very high abundance and diversity of trees. Most trees species found there contain unlike other savannah trees, large, broad leaves, but no spines. In most of the savannah, tree growth and survival are often limited by water availability and fires, and small, fine leaves are usually seen as adaptation with this. Contrary to this, kopjes contain soils with much higher water availability and lower fire risk, which might explain why trees growing there have such large and broad leaves. There are two

possible reasons why trees growing on kopjes generally do not contain spines: firstly, the benign growing conditions make it less important to protect tissue from being eaten and secondly, herbivore densities seem lower. These herbivores in turn might evade kopje because of the high abundance of predators (such as hyenas) found there. And the predators, in turn, may prefer to rest on kopjes because they act as a shade-rich environment and as high look-out points over the surrounding savannah. Concluding, we see that both community compositions of lower trophic levels and community compositions of higher trophic levels are important in understanding the assembly of a 'target community' under investigation. While lower trophic levels form resources, higher trophic levels form risks, and both resources and risks determine which species can or cannot live at a certain location.

In the two chapters afterwards (6,7) I explored how ecosystem-engineering affects communities. Ecosystem-engineering effects are (indirect) effects that organisms impose on other species through non-trophic processes. For example, beavers construct dams, termites and ants construct mounds, some plant species make the soil more acid and large terrestrial animals compact soils by trampling. By doing so, these organisms indirectly affect the presence and abundance of other species, and this process is called ecosystem engineering. Although ecosystem-engineering effects are ubiquitous, they are hardly considered in the literature on trait-based community assembly. I explored the effects on community assembly of two types of ecosystem-engineers: termites, which construct mounds with relatively 'benign' (high nutrient and moisture availability, low fire risk) microconditions, and ungulate herbivores, which compact soils in savannahs with their trampling behaviour and therefore create more 'stressful' conditions for plant growth. Tree species dominating on termite mounds indeed seemed more adapted to benign conditions than trees dominating the surroundings: most are evergreen, without spines and with large leaves, making them less adapted to drought, fire and herbivory. By trampling, ungulate herbivores compact soils and reduce water infiltration, and therefore we expected that trampling would act as a 'drought filter' for grass community assembly. Contrary to these expectations, grass species dominating on soils compacted by herbivores appeared to be no more drought-resistant than other grass species. However, their root traits and their fast, horizontal growth suggest grasses growing on compacted soils they might be much better than other grass species in recolonizing patches that have become bare after a severe drought. Concluding, we thus see that ecosystem-engineers affect community composition in savannahs, and that these shifts in communities can be at least partially explained by looking at the traits of the present species. However, when we investigated how trait *similarity* patterns differ between trees communities growing on termite mounds and in the surroundings, we did not find differences. Future studies could investigate more whether other ecosystem engineers do affect within-community trait distribution patterns.

In the last chapter (8) we studied firstly how the interplay of different community assembly processes affects within-community trait distributions, and secondly, vice versa: what we can learn from observed trait distributions about the relative contribution of different community assembly processes. To do that, we distinguished between three types of main processes, which are defined by their effect on trait distribution patterns: filtering processes, reducing possible trait range of co-occurring species (e.g. abiotic filtering or asymmetrical competition); limiting similarity processes, which limit the similarity of traits of co-occurring species (e.g. limiting similarity of shared resources or shared predator species); and stochastic processes, which are irrespective to trait values of species. We constructed simulation models which can differ in the relative contribution by which communities are assembled by these three processes. We then showed that in line with classical theory, (abiotic) filtering leads to trait underexpansion patterns. However, unlike predictions from classical theory, trait dispersion is not necessarily highest in communities solely shaped by limiting similarity processes, but even higher in communities that are shaped by a combination of limiting similarity *and* filtering processes. Using Bayesian inference, we then fitted these simulation models to trait distribution patterns of 20 tree communities, which were spread over a rainfall and fire gradient in Hluhluwe-iMfolozi Park. It appeared that generally communities were mostly shaped by stochastic

processes, less so by filtering and even less by limiting similarity processes. However, in communities of high rainfall and low fire frequency locations, limiting similarity is relatively more important.

Summarizing, while trophic and ecosystem engineering processes are almost completely ignored in the literature on trait-based community assembly, this thesis shows that these processes are in fact very important in shaping communities. Organisms affect the community assembly of other organisms not only by competing with them, but also by acting as food, acting as predator or by acting as an ecosystem-engineer. To my opinion, more appreciation of this fact and incorporation of these processes in other studies might highly advance the field of community assembly. At the same time, critics might say that trying to incorporate every little detail when studying community assembly might act counter-productive, obscuring us from seeing the bigger picture. Probably, the truth is somewhere in the middle: while 'case studies' on 'specific details' are important in demonstrating that a particular process is important, at the same time, we want to know *how* important this process is compared to other processes. In the chapter 8, we showed with a new modeling approach that it is possible to estimate the relative contribution of different 'groups of lumped processes' on community assembly, although more (experimental) follow-up studies are important to validate model outcomes and to get even more specific insights. I hope that in the future, a combination of modeling studies and much more experimental work will teach us to which extent communities are assembled by various different processes (e.g. abiotic filtering, competition, predation, facilitation, stochasticity, etc.) and how the relative contributions of these differ both within and between ecosystems.

# Nederlandse samenvatting

Groepen van lokaal bij elkaar voorkomende planten- of diersoorten, beter bekend als '(ecologische) gemeenschappen', zijn de uitkomst van voortdurende immigratie-, emigratie-, voortplantings- en uitstervingsgebeurtenissen. Tot op een zekere hoogte zijn deze gebeurtenissen toevalsprocessen: het is bijvoorbeeld ontzettend moeilijk, zo niet onmogelijk, om te voorspellen of een zaad dat door de wind verspreid wordt zich wel of niet in een bepaalde locatie zal vestigen. Tegelijkertijd is het zo dat al sinds wetenschappers begonnen met gemeenschapsecologie te bestuderen, zij ook erkennen dat processen met betrekking tot de ontwikkeling van gemeenschappen (gemeenschaps-ontwikkeling) deels deterministisch zijn: je kunt er zeker van zijn dat je in Afrikaanse savannes geen walvissen, mangrove bomen of pinguïns zult vinden, terwijl je wel grazende zoogdieren zult tegenkomen.

De reden waarom sommige soorten wel (potentieel) in een bepaalde locatie kunnen leven en andere niet, is omdat soorten van elkaar verschillen in 'functionele eigenschappen'. 'Eigenschappen' worden gedefinieerd als meetbare kenmerken van een organisme, zoals lichaamsgrootte, stofwisselingssnelheid, groeisnelheid en bladdikte. Ze worden als 'functioneel' beschouwd als ze leiden tot verschillen in 'prestaties' (bijvoorbeeld het hoeveelheid nageslacht of overleving) van organismen. Hoewel individuen *binnen* soorten vaak van elkaar verschillen in (functionele) eigenschappen, is de variatie van eigenschappen *tussen* soorten vaak nog groter. Dat is niet verrassend: diezelfde variatie van eigenschappen tussen soorten wordt gebruikt om soorten te determineren. Veel van die eigenschappen zijn niet alleen bepalend bij de determinatie van soorten, maar ook bij het bepalen in wat voor een omgeving een soort kan leven. Om bijvoorbeeld in een zeer droge omgeving te kunnen groeien moeten planten zuinig zijn met water. Een manier om dat te doen is door alleen zeer dikke (succulente) bladeren of helemaal géén bladeren (cactussen) te maken, waardoor de verdamping van water gereduceerd wordt. Hierdoor zul je in een woestijn alleen succulenten en cactusachtige plantensoorten vinden, maar geen plantensoorten met dunne bladeren. Dit wordt 'abiotische filteren' genoemd: aspecten van de abiotische (niet levende) omgeving (in dit voorbeeld: droogte) sluiten sommige soorten wel, maar andere soorten niet, uit om te leven op een bepaalde plek.

Vaak kunnen functionele eigenschappen dus gebruikt worden om te voorspellen in wat voor een omgeving een soort kan leven. Echter, als men alleen onderzoekt welke soorten wel of juist niet 'weggefilterd' worden in een bepaalde omgeving zal men nog steeds niet volledig gemeenschaps-ontwikkeling leren begrijpen. Soorten reageren namelijk niet alleen op hun abiotische omgeving, maar ook op interacties met andere soorten. Een type interactie dat vaak bestudeerd wordt is competitie. Als twee soorten, zoals bijvoorbeeld de rode (*Sciurus vulgaris*) en grijze eekhoorn (*Sciurus carolinensis*) hetzelfde voedsel eten, dan gebeurt het vaak dat één van de twee soorten (in dit voorbeeld: de grijze eekhoorn) efficiënter is in het verzamelen van voedsel dan de ander. Daardoor zal de 'zwakkere concurrent' (de rode eekhoorn in dit voorbeeld) weggeconcurreerd worden. Vaak kunnen functionele eigenschappen gebruikt worden om te voorspellen in hoeverre soorten met elkaar zullen concurreren: soorten concurreren als ze (deels) hetzelfde voedsel eten, terwijl dieetkeuze vaak door functionele eigenschappen bepaald wordt. In het voorbeeld van de rode en de grijze eekhoorn is het zo dat het gemeenschappelijke bezit van scherpe snijtanden en vergelijkbare verteringsstelsels maakt dat beide soorten zich specialiseren op het eten van zaden en noten. Daardoor concurreert de meer efficiënte grijze eekhoorn de rode eekhoorn weg.

Als we kijken naar abiotische filters en competitie en naar hoe de uitkomst van die processen door functionele eigenschappen bepaald wordt, dan zien we iets grappigs: terwijl soorten vaak op elkaar moeten lijken (gelijk moeten zijn) om in hetzelfde (abiotische) biotoop voor te kunnen komen, moeten ze tegelijkertijd van elkaar verschillen in eigenschappen met betrekking tot dieet (ongelijk zijn) om samen in dezelfde gemeenschap voor te kunnen komen. Dit conceptuele idee wordt van oudsher gebruikt in studies naar gemeenschapsontwikkeling om de aanwezigheid van drie verschillende gemeenschapsontwikkelings-processen te bestuderen. Als (a) waardes van (kwantificeerbare) eigenschappen van lokaal bij elkaar voorkomende soorten een minder grote

spreidingsbreedte vertonen dan een 'willekeurige' groep van even veel soorten (een patroon dat 'onderexpansie van eigenschappen' wordt genoemd), dan concludeert men meestal dat dit door abiotische filters komt. Als (b) de waardes van functionele eigenschappen van lokaal bij elkaar voorkomende soorten meer regelmatig van elkaar afliggen dan die van een 'willekeurige' groep van even veel soorten (een patroon dat 'overspreiding van eigenschappen' wordt genoemd), dan concludeert men meestal dat dit het resultaat is van competitie tussen soorten. In het laatste geval (3) dat zowel de spreidingsbreedte als de spreidingsregelmaat van functionele eigenschappen van bij elkaar voorkomende soorten hetzelfde zijn als in een 'willekeurige' (random) gemeenschap met hetzelfde aantal soorten, dan concludeert men vaak dat toeval het meest bepalend is in de ontwikkeling van ecologische gemeenschappen.
Deze methode om de processen betrokken bij de ontwikkeling van ecologische gemeenschappen te bestuderen is intuïtief simpel, wat waarschijnlijk de voornaamste reden is waarom deze methode de afgelopen jaren erg populair is geworden. Echter, de aanpak wordt ook becriticeerd. Een eerste punt van kritiek is dat deze aanpak een heleboel ecologische processen negeert die vaak wel belangrijk zijn bij het ontwikkelen van ecologische gemeenschappen: onder andere trofische interacties (eten en gegeten worden) tussen soorten en 'ecosysteem ingenieurende processen' zoals de bouw van beverdammen. Ook is het zo dat er meestal meerdere ecologische processen tegelijkertijd betrokken zijn bij het vormen van ecologische gemeenschappen: een gemeenschap wordt meestal gevormd door *zowel* abiotische filters, competitie *áls* toevalsprocessen, niet door óf abiotische filters, óf competitie, óf toevalsprocessen. In dit proefschrift heb ik met deze twee punten van kritiek in mijn achterhoofd gemeenschapsontwikkeling bestudeerd: in Afrikaanse savannes heb ik onderzocht in hoeverre het in acht nemen van (1) trofische interacties en (2) ecosystem ingenieurende processen ons beeld van gemeenschapsontwikkeling verandert. Ook heb ik (3) onderzocht hoe de *interactie* tussen verschillende ecologische processen de spreiding van functionele eigenschappen van bij elkaar voorkomende soorten bepaalt.

In de eerste vier hoofdstukken (1,2,3,4) onderzocht ik de effecten van trofische interacties op gemeenschapsontwikkeling en de spreiding van functionele eigenschappen in ecologische gemeenschappen. In savannes van Hluhluwe-iMfolozi Park (HiP) in Zuid Afrika heb ik onderzocht hoe de gemeenschapsontwikkeling van twee verschillende trofische niveaus, grassen en (plantenetende) sprinkhanen, beïnvloed worden door (1) begrazing (door b.v. neushoorns, zebra's, buffels en antilopen), (2) milieu-gradiënten en (3) de gemeenschaps-samenstelling van het andere trofische niveau.
Het bleek dat grazende zoogdieren mozaïeken van korte en lage vegetatie creëren, waarbij de korte vegetatie gedomineerd wordt door door klein-bladige grassoorten, terwijl de hogere vegetatie gedomineerd wordt door groot-bladige grassoorten. Daardoor verhogen grazende zoogdieren op grotere ruimtelijke schalen de soortenrijkdom van grassen. Sprinkhanen gemeenschappen worden ook beïnvloed door begrazing: begraasde plekken bevatten andere, meestal kleinere, sprinkhanensoorten dan onbegraasde plekken. Echter, de soortenrijkdom van sprinkhanen wordt *niet* beïnvloed door begrazing. Misschien komt dat doordat positieve effecten van begrazing, zoals meer variatie van voedselbronnen (meer grassoorten) worden gecompenseerd door negatieve effecten, zoals een minder groot aanbod van voedsel (minder biomassa aan grassen). Verder bleek dat begrazing geen invloed heeft op de spreiding van functionele eigenschappen van bij elkaar voorkomende gras- of sprinkhanensoorten. Dit was tamelijk verrassend: men zou kunnen denken dat door toegenomen plantenstress (door het opeten van plantendelen) in begraasde gebieden, grazers soorten met bepaalde eigenschappen wegfilteren en daardoor voor onderexpansie van planteneigenschappen zorgen, die vervolgens 'gekopieerd' worden naar herbivore sprinkhaangemeenschappen. Mogelijk is het zo dat het vegetatiemozaïek dat door grazers gecreëerd wordt dit voorkómt: op een grotere schaal vindt je zowel korte vegetatie met soorten tolerant die begrazing tolereren, als hoge vegetatie met soorten die niet tolerant zijn voor begrazing.
Hoewel expansie patronen van functionele eigenschappen in sprinkhaan- en gras gemeenschappen niet reageerden op begrazing, reageerden ze wel op mileugradiënten: over het algemeen vertoonden

grassen eigenschappen vooral onderexpansie in gebieden met weinig regen en veel vuur, zoals klassieke theoriën voorspellen. Dit was niet het geval voor sprinkhanen: expansie patronen van sprinkhaan-gemeenschappen reageerden niet op milieugradiënten, maar wel op de expansiepatronen van planteneigenschappen. Dit laat zien dat de spreiding van kenmerken van één trofisch niveau 'gekopieerd' kunnen worden naar een ander (in dit geval: hoger) trofisch niveau én dit benadrukt dus hoe belangrijk trofische interacties zijn om gemeenschaps-ontwikkeling te begrijpen. Een inspectie van de al bestaande, relevante literatuur over dit onderwerp liet zien dat veel van de waarnemingen van de hierboven beschreven studies overeenkomen met die van ander onderzoek. Over het algemeen zijn de effecten van begrazing op de soortenrijkdom van planten positiever (of minder negatief) dan de effecten op de soortenrijkdom van geleedpotigen (zoals insecten). Ook is het zo dat studies vaak laten zien dat begrazing positief uitpakt voor kleinere geleedpotigen, maar niet voor grotere soorten. Mogelijk komt dit doordat kleinere soorten minder voedsel (biomassa aan planten) nodig hebben, minder kwetsbaar zijn voor predatie in een korte (of afwezige) vegetatie, een hogere temperatuur nodig hebben om te groeien, of door een combinatie van deze factoren.
In het laatste hoofdstuk over de rol van trofische interacties in gemeenschaps-ontwikkeling wordt laten zien dat in de Serengeti (Tanzania) rotsformaties, ook bekend als kopjes, mini-eilandjes vormen in de savanne, met een behoorlijk hoge dichtheid en diversiteit van bomen. De meeste bomen die op kopjes groeien hebben grote bladeren, maar zijn niet stekelig, in tegenstelling tot de meeste andere bomen van de savanne. In het grootste gedeelte van de savanne wordt de groei en de overleving van bomen gelimiteerd door een gebrek aan water en door vuur. Kleine bladeren worden vaak gezien als een aanpassing hierop. Kopjes vormen plekken met een (zeer lokaal) hogere beschikbaarheid van water en een kleiner risico op branden. Dit kan mede verklaren waarom de bomen op kopjes relatief grote bladeren hebben. Er zijn twee mogelijke redenen waarom bomen op kopjes vaak niet stekelig zijn: ten eerste is het zo dat vanwege de gunstige omstandigheden voor een hoge groeisnelheid op kopjes, het minder belangrijk is om je als boom tegen vraat te verdedigen. Ten tweede is het zo dat de dichtheden van bladetende zoogdieren er lager is. Deze zoogdieren vermijden kopjes mogelijk vanwege de waargenomen hogere dichtheden van roofdieren (zoals hyena's). En de roofdieren prefereren hun tijd door te bringen op kopjes mogelijk omdat deze schaduwrijk zijn en een goed uitzicht bieden op de rest van de savanne.
Concluderend zien we dat zowel de gemeenschappen van hogere trofische niveaus als die van lagere trofische niveaus belangrijk zijn om de ontwikkeling van een 'focus-gemeenschap' te begrijpen. Terwijl lagere trofische niveaus voedsel vormen, vormen hogere trofische niveaus overlevingsgevaren. Zowel voedsel als gevaren bepalen of een soort wel of niet in een bepaalde locatie kan leven.

In de volgende twee hoofdstukken (6,7) onderzocht ik hoe het proces 'ecosystem-ingeniëren' gemeenschappen beïnvloed. Ecosysteem ingenieurs-effecten zijn (indirecte) effecten die organismen op andere organismen hebben vanwege niet-trofische processen. Wat voorbeelden: bevers bouwen dammen, termieten bouwen heuvels, sommige planten verzuren de grond en grote zoogdieren drukken de grond samen. Daarmee hebben deze organismen indirecte effecten op de aanwezigheid en dichtheid van andere soorten, wat 'ecosysteem ingeniëren' genoemd wordt. Hoewel ecosysteem-ingenieurs effecten overal voorkomen worden ze nauwelijks beschouwd in de literatuur over gemeenschaps-ontwikkeling.
Ik onderzocht de effecten van twee verschillende type ecosysteem-ingenieurs processen op gemeenschaps-ontwikkeling: ten eerste de effecten van heuvel bouwende termieten die hun omgeving meer nutriëntenrijk, vochtig, minder vatbaar voor brand en daarmee meer 'comfortabel' voor planten maken. Ten tweede onderzocht ik de effecten van grazende zoogdieren, die door rond te lopen in de savanne de aarde samendrukken, droger maken en daardoor hun omgeving meer 'stressvol' voor planten maken. Bomen die op termietenheuvels groeiden leken inderdaad meer aangepast op 'aantrekkelijke' omgevingen dan bomen van 'standaard' savanne: ze waren vaker overblijvend, zonder stekels en met grote bladeren, wat ze minder aangepast maakt op droogte, vuur en herbivorie. Van omgevingen die veel bezocht worden door grazende zoogdieren verwachtten we dat de aarde

platgetrapt is en daardoor compact en droog, zodat zwaar begraasde omgevingen zich als een 'droogte-filter' voor planten gedragen. Toch bleek het niet zo te zijn dat grassen kenmerkend voor sterk begraasde locaties beter tegen droogte zijn aangepast dan andere grassoorten. Wel bleken grassen kenmerkend van begraasde plekken dunne wortels te hebben en relatief snel te groeien, wat suggereert dat deze soorten, nadat ze deels afsterven vanwege een droogte, wellicht ook weer snel terug kunnen groeien.
Concluderend zien we dat ecosysteem-ingenieurs invloed hebben op ecologische gemeenschappen in savannes en dat deze invloeden ten minste tot op een zekere hoogte door functionele eigenschappen van soorten verklaard kunnen worden. Echter, toen ik onderzocht in hoeverre de (on)gelijkbaarheid van eigenschappen van bij elkaar voorkomende soorten bepaald wordt door ecosysteem-ingenieurs vond ik geen duidelijke patronen. Toekomstige studies kunnen hier meer naar kijken.

In het laatste hoofdstuk (8) bestudeerde ik eerst hoe de interactie tussen verschillende gemeenschaps-ontwikkelingsprocessen de spreiding van functionele eigenschappen in gemeenschappen beïnvloed. Ten tweede onderzocht ik het tegenovergestelde: wat we over gemeenschaps-ontwikkelingen kunnen leren door te kijken naar waargenomen verspreidingspatronen in functionele eigenschappen van bij elkaar voorkomende soorten. Om dat te doen heb ik eerst onderscheid gemaakt tussen drie verschillende typen ecologische processen, ingedeeld op hun effect op verspreidingspatronen van eigenschappen. Ten eerste zijn er filter processen, die de spreidingsbreedte van functionele eigenschappen binnen gemeenschappen limiteren (b.v. vanwege abiotische filters of assymetrische lichtcompetitie). Ten tweede zijn er limiterende gelijkheidsprocessen, die de gelijkheid van eigenschappen van bij elkaar voorkomende soorten limiteren (b.v. vanwege competitie om voedsel). Ten derde zijn er toevalsprocessen, die onafhankelijk zijn van de eigenschappen van organismen. Ik ontwikkelde simulatiemodellen die het relatieve belang van die drie processen in het vormen van ecologische gemeenschappen schatten. Daarna liet ik zien dat, zoals klassieke theoriën ook suggereren, filter processen leiden tot onderexpansie van soortseigenschappen. Echter, in tegenstelling tot wat klassieke theoriën zeggen, is de spreiding van eiegnschappen van bij elkaar voorkomende soorten niet noodzakelijkerwijs het hoogst in gemeenschappen die enkel door competitie (limiterende gelijkheid processen) gevormd worden: de spreiding van functionele eigenschappen is het hoogst in gemeenschappen die zowel door filter processen als door limiterende gelijkheidsprocessen gevormd worden. Met Bayesiaanse methodes hebben we daarna de uitkomsten van simulatiemodellen 'gefit' met waargenomen eigenschappen-verspreidings patronen van twintig verschillende boomgemeenschappen uit Zuid-Afrika, afkomstig uit verschillende locaties over een regen- en vuurgradiënt. Het bleek dat toevalsprocessen het belangrijkst zijn bij het vormen van deze gemeenschappen, terwijl filter processen en limiterende gelijkheidsprocessen minder belangrijk zijn. Echter, in plekken met veel regen en weinig vuur bleken limiterende gelijkheidsprocessen relatief belangrijker bij het vormen van boom-gemeenschappen.

Concluderend zien we dat hoewel trofische en ecosysteem-ingenieurs processen bijna compleet genegeerd worden in studies naar de ontwikkeling van ecologische gemeenschappen, deze processen onmisbaar zijn om gemeenschaps-ontwikkeling volledig te leren begrijpen. Organismen beïnvloeden andere organismen niet alleen door middel van concurrentie, maar ook door als voedsel te dienen, als roofdier of als een ecosysteem-ingenieur. Naar mijn mening zou een grotere waardering voor dit feit en het invoegen van deze processen in andere studies naar gemeenschaps-ontwikkeling een belangrijke stap voortwaarts voor dit onderzoeksveld betekenen. Tegelijkertijd kunnen critici natuurlijk ook zeggen dat het in acht nemen van elk klein detail bij het onderzoeken van de ontwikkeling van ecologische gemeenschappen nauwelijks nuttig is: details kunnen ons afleiden van 'het grotere geheel'. De werkelijkheid zit waarschijnlijk ergens in het midden: hoewel 'case-studies' naar 'specifieke details' belangrijk zijn om de relevantie van een bepaald proces aan te tonen, willen we tegelijkertijd weten hoe belangrijk een bepaald proces is in het vormen van ecologische gemeenschappen *ten opzicht van* andere processen. In hoofdstuk 8 heb ik laten zien dat het mogelijk is om met simulatiemodellen om een ruw beeld te krijgen van het relatieve belang van verschillende

processen op het vormen van ecologiscge gemeenschappen, hoewel (experimentele) vervolgstudies nodig zijn om conclusies te valideren en om specifiekere inzichten te krijgen. Ik hoop dat in de toekomst een combinatie van simulatiestudies en meer experimentele studies ons zal leren in hoeverre ecologische gemeenschappen gevormd worden door verschillende processen (zoals abiotische filters, competitie, predatie, facilitatie, toevalsprocessen, etc.) en hoe het relatieve belang van deze processen verschilt binnen en tussen ecosystemen.

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